-1-

3-ARYLOXY/THIO-3-SUBSTITUTED PROPANAMINES AND THEIR USE IN INHIBITING SEROTONIN AND NOREPINEPHRINE REUPTAKE

This invention relates to 3-aryloxy/thio-3-substituted propanamines, and to their use in inhibiting serotonin and norepinephrine reuptake.

5

Serotonin (5HT) has been implicated in the aetiology of many disease states and has been found to be of importance in mental illnesses, depression, anxiety, schizophrenia, eating disorders, obsessive compulsive disorder (OCD) and migraine. Indeed many currently used treatments of these disorders are thought to act by modulating serotonergic tone.
10 During the last decade, multiple serotonin receptor subtypes have been characterised. This has led to the realisation that many treatments act *via* the serotonergic system, such as selective serotonin reuptake inhibitor (SSRI) antidepressants which increase serotonin transmission, such as, for example, the hydrochloride salt of fluoxetine.

15 Drugs that exert their main action on the norepinephrinergic system have been available for some time, however their lack of selectivity made it difficult to determine specific clinical effects produced by a selective action on norepinephrine reuptake. Accumulating evidence indicates that the norepinephrinergic system modulates drive and energy, whereas the serotonergic system modulates mood. Thus norepinephrine appears to play an
20 important role in the disturbances of vegetative function associated with affective, anxiety and cognitive disorders. Atomoxetine hydrochloride is a selective inhibitor of norepinephrine, and is currently marketed for the treatment of attention deficit hyperactivity disorder (ADHD).

25 Norepinephrine and serotonin receptors are known to interact anatomically and pharmacologically. Compounds that affect only serotonin have been shown to exhibit modulatory effects on norepinephrine, pointing toward an important relationship between the two neurotransmitter systems.

30 Duloxetine, (+)-*N*-methyl-3-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride, inhibits the reuptake of both norepinephrine and serotonin, and is currently under

-2-

development for the treatment of depression and urinary incontinence. The compound
duloxetine was disclosed in US Patents 5,023,269 and 4,956,388.

US patent number 4,018,895 describes aryloxyphenyl propanamine compounds including
compunds of the formula

$$R\text{-}O\text{-}CH\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}CH_3$$
$$|$$
$$C_6 H_5$$
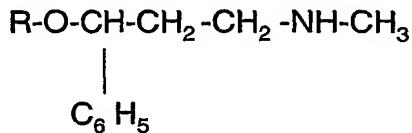
Where R is, for example, phenyl, substituted phenyl, tolyl or anisyl. The compounds
block the uptake of various physiologically active monoamines including serotonin,
norepinephrine and dopamine. Some of the compounds are selective to one of the
monoamines and others have multiple activity. The compounds are indicated as
psychotropic agents. Some are also antagonists of apomorphine and/or reserpine.

WO 00/02551 describes *inter alia* 3-aryloxy-3-substituted propanamines which are active
at the NMDA receptor and serotonin reuptake site.

WO 96/09288 describes indole derivatives which are active at the 5HT receptor. The 5-
membered ring portion of the indole moiety is further substituted by one of a number of
amine functional groups.

WO 01/62714 discloses phenylheteroalkylamine derivatives which are inhibitors of nitric
oxide synthase. WO 03/011831 discloses heteroarylheteroalkylamine derivatives which
are inhibitors of nitric oxide synthase.

WO 02/094262 discloses heteroaryloxy 3-substituted propanamines as serotonin and
norepinephrine reuptake inhibitors.

The present invention provides novel 3-aryloxy/thio-3-substituted propanamines which
are potent inhibitors of both serotonin and norepinephrine reuptake. Preferred compounds
of the present invention exihibit (i) greater potency of inhibition of the serotonin and/or

-3-

norepinephrine transporters, and/or (ii) improved selectivity of inhibition of the serotonin and/or norepinephrine transporters relative to the dopamine transporter, and/or (iii) improved ADME properties (e.g. reduced tendency to act as a substrate and/or inhibitor for the enzyme Cytochrome P450 2D6), and/or (iv) improved acid stability, as compared to known inhibitors of both serotonin and norepinephrine reuptake.

According to the present invention there is provided a compound of formula I:
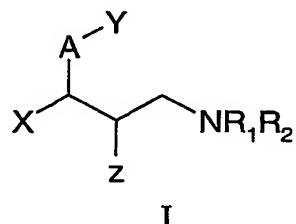
I

wherein

A is selected from -O- and -S-;

X is selected from C_2-C_8 alkyl, C_2-C_8 alkenyl, C_3-C_8 cycloalkyl and C_4-C_8 cycloalkylalkyl, each of which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, -CF_3, -CN and -$CONH_2$;

Y is selected from phenyl, naphthyl, dihydrobenzothienyl, benzothiazolyl, benzoisothiazolyl, quinolyl, isoquinolyl, naphthyridyl, thienopyridyl, indanyl, 1,3-benzodioxolyl, benzothienyl, indolyl and benzofuranyl, each of which may be optionally substituted with up to 4 or, where possible, 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, nitro, acetyl, -CF_3, -SCF_3 and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C_1-C_4 alkyl;

Z is selected from H, OR_3 or F, wherein R_3 is selected from H, C_1-C_6 alkyl and phenyl C_1-C_6 alkyl;

R_1 and R_2 are each independently H or C_1-C_4 alkyl;

and pharmaceutically acceptable salts thereof,

with the proviso that, when Z is H, then Y may not be optionally substituted phenyl or optionally substituted naphthyl.

-4-

The compounds of the present invention are potent and (preferably) selective inhibitors of serotonin and norepinephrine reuptake.

5   In one group of compounds according to the present invention, A is -O-.

In another group of compounds according to the present invention, A is -S-.

Preferably, one of R_1 and R_2 is H.

10

R_1 and R_2 may both be H. Alternatively, one of R_1 and R_2 may be H while the other is C_1-C_4 alkyl, for example C_1-C_3 alkyl. Preferably, one of R_1 and R_2 is H and the other is methyl.

15   It will be appreciated that a compound of formula I will possess at least one or, when Z is not H, at least two chiral centres. Where a structural formula does not specify the stereochemistry at one or more chiral centres, it encompasses all possible stereoisomers and all possible mixtures of stereoisomers (including, but not limited to, racemic mixtures) which may result from stereoisomerism at each of the one or more chiral
20   centers.

In one embodiment of the present invention, the compound possesses the stereochemistry defined in formula II

II
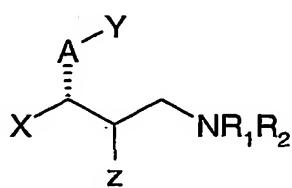
25

In another embodiment of the present invention, the compound possesses the stereochemistry defined in formula III

$$\text{X}-\underset{\underset{\text{Z}}{|}}{\overset{\overset{\text{Y}}{|}}{\text{A}}}-\text{NR}_1\text{R}_2$$

III
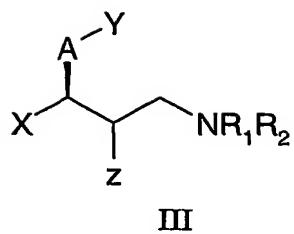
In another embodiment of the present invention, Z is H.

In another embodiment of the present invention, X is C_2-C_8 alkyl which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-S(O)$_n$- where n is 0, 1 or 2, -CF$_3$, -CN and -CONH$_2$. In this embodiment, X is preferably selected from ethyl, n-propyl, i-propyl, n-butyl, i-butyl, n-pentyl, i-pentyl, neopentyl, 3,3-dimethylbutyl and 2-ethylbutyl, each of which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy and -CF$_3$. More preferably, X is selected from n-propyl, i-propyl, n-butyl and i-butyl.

In another embodiment of the present invention, X is C_2-C_8 alkenyl which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-S(O)$_n$- where n is 0, 1 or 2, -CF$_3$, -CN and -CONH$_2$. In this embodiment, X is preferably 2-methyl-2-propenyl.

In another embodiment of the present invention, X is C_4-C_8 cycloalkylalkyl which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-S(O)$_n$- where n is 0, 1 or 2, -CF$_3$, -CN and -CONH$_2$. In this embodiment, X is preferably selected from cyclohexylmethyl and cyclopropylmethyl.

In another embodiment of the present invention (when Z is not H), Y is phenyl optionally substituted with up to 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-S-, -CF$_3$, and -SCF$_3$. In this embodiment, Y is preferably

phenyl optionally substituted with up to 2 substituents each independently selected from F, Cl, Br, I, Me, Et, OMe, SMe, -CF$_3$, and -SCF$_3$. More preferably, Y is selected from 2-methylphenyl, 2-ethylphenyl, 2-chlorophenyl, 2-bromophenyl, 2-iodophenyl, 3-methylphenyl, 3-methoxyphenyl, 3-chlorophenyl, 3-fluorophenyl, 3-chlorophenyl, 3-trifluoromethylphenyl, 3-methylsulfanylphenyl, 4-methylphenyl, 4-chlorophenyl, 4-trifluoromethylsulfanylphenyl, 4-fluoro-2-methylphenyl, 3,5-dichlorophenyl and 2,4-dichlorophenyl.

In another embodiment of the present invention (when Z is not H), Y is naphthyl optionally substituted with up to 5 substituents each independently selected from halo, C$_1$-C$_4$ alkyl, C$_1$-C$_4$ alkoxy, C$_1$-C$_4$ alkyl-S(O)$_n$- where n is 0, 1 or 2, nitro, acetyl, -CF$_3$, -SCF$_3$ and cyano. In this embodiment, Y is preferably unsubstituted naphthyl or naphthyl which is mono-substituted with a substituent selected from halo, C$_1$-C$_4$ alkyl and -CF$_3$. When the naphthyl group is substituted, the substituent is preferably located at the 4-position of the naphthyl ring. The preferred point of attachment of the optionally substituted naphthyl group to the —O- or —S- atom is attachment at the 1 position. More preferably, Y is selected from naphth-1-yl, 4-trifluoromethyl-naphth-1-yl, 4-chloro-naphth-1-yl, 4-fluoro-naphth-1-yl, 4-methyl-naphth-1-yl and 4-bromo-naphth-1-yl.

In another embodiment of the present invention, Y is benzofuranyl, benzothiazolyl, benzoisothiazolyl or indolyl each of which may be optionally substituted with up to 4 or, where possible, 5 substituents each independently selected from halo, C$_1$-C$_4$ alkyl, C$_1$-C$_4$ alkoxy, C$_1$-C$_4$ alkyl-S(O)$_n$- where n is 0, 1 or 2, nitro, acetyl, -CF$_3$, -SCF$_3$ and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C$_1$-C$_4$ alkyl. In this embodiment, Y is preferably benzofuranyl, benzoisothiazolyl or indolyl each of which may be optionally mono-substituted with Me; and when Y is indolyl it may be substituted or further substituted by an N-methyl substituent. In this embodiment, the preferred point of attachment of the group Y to the —O- or —S- atom is attachment at the 4 or 7 position. When Y is N-substituted indolyl, the substituent is preferably Me. More preferably, Y is selected from benzofuran-7-yl, 2-methyl-benzofuran-7-yl, indol-7-yl, N-methyl-indol-7-yl, indol-4-yl, N-methyl-indol-4-yl, benzoisothiazol-7-yl, benzoisothiazol-4-yl, 7-methyl-benzoisothiazol-7-yl.

-7-

In another embodiment of the present invention, Y is benzothienyl optionally substituted with up to 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, nitro, acetyl, -CF_3, -SCF_3 and cyano. In this embodiment, Y is preferably benzothienyl optionally substituted with up to 2 substituents each independently selected from halo, C_1-C_4 alkyl, -CF_3 and cyano. In this embodiment, the preferred point of attachment of the group Y to the –O- or –S- atom is attachment at the 4, 5, 6 or 7 position. More preferably, Y is selected from benzothien-4-yl, 5-fluoro-benzothien-4-yl, 7-methyl-benzothien-4-yl, 7-fluoro-benzothien-4-yl, 7-cyano-benzothien-4-yl, 2-fluoro-benzothien-4-yl, 2-bromo-benzothien-4-yl, 3-methyl-benzothien-4-yl, benzothien-5-yl, benzothien-6-yl, 4-trifluoromethyl-benzothien-6-yl, benzothien-7-yl, 6-fluoro-benzothien-7-yl, 6-chloro-benzothien-7-yl, 4-methyl-benzothien-7-yl, 4-bromo-benzothien-7-yl, 4-cyano-benzothien-7-yl, 4-fluoro-benzothien-7-yl, 2-methyl-benzothien-7-yl, 2-cyano-benzothien-7-yl, 2-fluoro-benzothien-7-yl, 2-bromo-benzothien-7-yl, 5-fluoro-benzothien-7-yl, 4-fluoro-3-methyl-benzothien-7-yl and 3-chloro-4-fluoro-benzothien-7-yl.

In another embodiment of the present invention, Y is quinolyl or isoquinolyl each of which may be optionally substituted with up to 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, nitro, acetyl, -CF_3, -SCF_3 and cyano. In this embodiment, Y is preferably quinolyl or isoquinolyl each of which may be optionally mono-substituted with a halogen atom. In this embodiment, the preferred point of attachment of the group Y to the –O- or –S- atom is attachment at the 5 or 8 position. More preferably, Y is selected from quinol-8-yl, 5-chloro-quinol-8-yl and isoquinol-5-yl.

In another embodiment of the present invention, Y is thienopyridyl which may be optionally substituted with up to 4 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, nitro, acetyl, -CF_3, -SCF_3 and cyano. In this embodiment, Y is preferably unsubstituted thieno-[3,2-b]pyridyl or unsubstituted thieno-[3,2-c]pyridyl. In this embodiment, the preferred point of attachment of the group Y to the –O- or –S- atom is attachment at the 7 position.

The present invention also provides sub-groups of compounds of formula I or II or III:

I   II   III

wherein

A is selected from -O- and -S-;

X is selected from C_2-C_8 alkyl, C_2-C_8 alkenyl, C_3-C_8 cycloalkyl and C_4-C_8 cycloalkylalkyl, each of which may be optionally substituted with up to 3 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, -CF_3, -CN and -$CONH_2$;

Y is selected from dihydrobenzothienyl, benzothiazolyl, benzoisothiazolyl, quinolyl, isoquinolyl, naphthyridyl, thienopyridyl, indanyl, 1,3-benzodioxolyl, benzothienyl, indolyl and benzofuranyl, each of which may be optionally substituted with up to 4 or, where possible, 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, nitro, acetyl, -CF_3, -SCF_3 and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C_1-C_4 alkyl;

Z is selected from H, OR_3 or F, wherein R_3 is selected from H, C_1-C_6 alkyl and phenyl C_1-C_6 alkyl;

R_1 and R_2 are each independently H or C_1-C_4 alkyl;

and pharmaceutically acceptable salts thereof.

The present invention also provides sub-groups of compounds of formula I or II or III:

wherein

A is selected from -O- and -S-;

X is selected from C_2-C_8 alkyl, C_2-C_8 alkenyl and C_4-C_8 cycloalkylalkyl, each of which may be optionally substituted with up to 3 substituents each independently selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, -CF_3, -CN and -$CONH_2$;

Y is selected from dihydrobenzothienyl, benzothiazolyl, benzoisothiazolyl, quinolyl, isoquinolyl, naphthyridyl, thienopyridyl, indanyl, 1,3-benzodioxolyl, benzothienyl, indolyl and benzofuranyl, each of which may be optionally substituted with up to 4 or, where possible, 5 substituents each independently selected from halo, C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 alkyl-$S(O)_n$- where n is 0, 1 or 2, -CF_3, -SCF_3 and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C_1-C_4 alkyl;

Z is selected from H, OR_3 or F, wherein R_3 is selected from H, C_1-C_6 alkyl and phenyl C_1-C_6 alkyl;

R_1 and R_2 are each independently H or C_1-C_4 alkyl;

and pharmaceutically acceptable salts thereof.

The present invention also provides sub-groups of compounds of formula I or II or III:
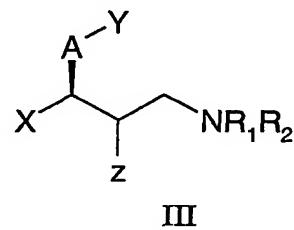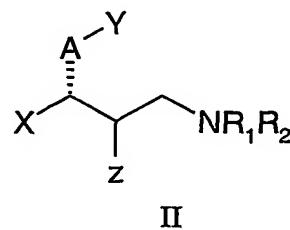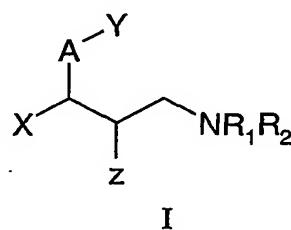
wherein

A is -O-;

X is selected from C_2-C_8 alkyl, and C_4-C_8 cycloalkylalkyl;

Y is selected from dihydrobenzothienyl, benzothiazolyl, benzoisothiazolyl, quinolyl, isoquinolyl, naphthyridyl, thienopyridyl, benzothienyl, indolyl and benzofuranyl, each of which may be optionally substituted with up to 4 or, where possible, 5 substituents each

5   independently selected from halo, C_1-C_4 alkyl, -CF_3, -SCF_3 and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C_1-C_4 alkyl;

Z is H;

R_1 and R_2 are each independently H or C_1-C_4 alkyl;

10   and pharmaceutically acceptable salts thereof

The present invention also provides sub-groups of compounds of formula I or II or III:

I   II   III
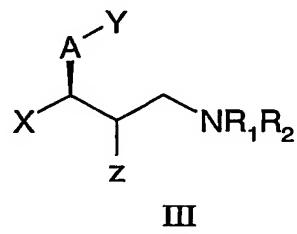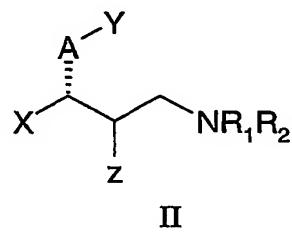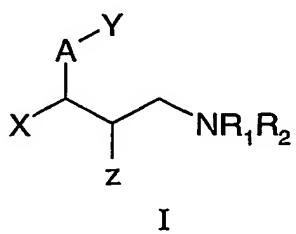
15   wherein

A is -O-;

X is selected from ethyl, n-propyl, i-propyl, n-butyl, i-butyl, n-pentyl, i-pentyl, neopentyl, 3,3-dimethylbutyl, 2-ethylbutyl, cyclohexylmethyl and cyclopropylmethyl;

Y is selected from benzothiazolyl, benzoisothiazolyl, quinolyl, isoquinolyl, naphthyridyl,

20   thienopyridyl, benzothienyl, indolyl and benzofuranyl, each of which may be optionally substituted with up to 2 substituents each independently selected from halo, methyl, -CF_3, -SCF_3 and cyano; and when Y is indolyl it may be substituted or further substituted by an N-substituent selected from C_1-C_4 alkyl;

Z is H;

25   R_1 is H and R_2 is Me;

and pharmaceutically acceptable salts thereof.

The present invention also provides sub-groups of compounds of formula I or II or III:

I	II	III

wherein

A is -O-;

X is selected from n-propyl, i-propyl, n-butyl, i-butyl, and cyclohexylmethyl;

Y is benzothienyl;

Z is H;

R_1 is H and R_2 is Me;

and pharmaceutically acceptable salts thereof.

In the present specification the term "C_2-C_8 alkyl" means a monovalent unsubstituted saturated straight-chain or branched-chain hydrocarbon radical having from 2 to 8 carbon atoms.

In the present specification the term "C_2-C_8 alkenyl" means a monovalent unsubstituted unsaturated straight-chain or branched-chain hydrocarbon radical having from 2 to 8 carbon atoms.

In the present specification the term "C_3-C_8 cycloalkyl" means a monovalent unsubstituted saturated cyclic hydrocarbon radical having from 3 to 8 carbon atoms.

In the present specification the term "C_4-C_8 cycloalkylalkyl" means a monovalent unsubstituted saturated cyclic hydrocarbon radical having from 3 to 7 carbon atoms linked to the point of substitution by a divalent unsubstituted saturated straight-chain or branched-chain hydrocarbon radical having at least 1 carbon atom.

In the present specification the term "halo" or "halogen" means F, Cl, Br or I.

-12-

In the present specification the term "C_1-C_4 alkoxy" means a monovalent unsubstituted saturated straight-chain or branched-chain hydrocarbon radical having from 1 to 4 carbon atoms linked to the point of substitution by an O atom.

5   In the present specification the term "phenyl C_1-C_6 alkyl" means a monovalent phenyl radical linked to the point of substitution by a divalent unsubstituted saturated straight-chain or branched chain hydrocarbon radical having from 1 to 6 carbon atoms.

In the above definitions, similar terms specifying different numbers of C atoms take an
10   analogous meaning.

In   the   present   specification   the   term   "dihydrobenzothienyl"   includes   2,3-dihydrobenzothienyl and 1,3-dihydrobenzothienyl. 2,3-dihydrobenzothienyl is preferred.

15   In the present specification the term "benzoisothiazolyl" includes 1,2-benzoisothiazolyl and 2,1-benzoisothiazolyl. 1,2-benzoisothiazolyl is preferred.

In the present specification the term "naphthyridyl" includes 1,5-, 1,6-, 1,7- and 1,8-naphthyridyl. 1,7- naphthyridyl is preferred.
20

In the present specification the term "thienopyridyl" includes thieno-[2,3-b]pyridinyl, thieno-[2,3-c]pyridinyl, thieno-[3,2-c]pyridinyl and thieno-[3,2-b]pyridinyl. Thieno-[3,2-b]pyridinyl and thieno-[3,2-c]pyridinyl are preferred.

25   In the present specification the term "benzothienyl" includes benzo[b]thienyl and benzo[c]thienyl. Benzo[b]thienyl is preferred.

In the present specification the term "benzofuranyl" includes 1-benzofuranyl and isobenzofuranyl. 1-benzofuranyl is preferred.
30

In the present specification the abbreviation "Ace-Cl" means α-chloroethyl chloroformate.

-13-

In the present specification the abbreviation "PS-DIPEA" means polymer-supported diisopropylethylamine.

The present invention also provides processes for producing a compound of formula I above or a pharmaceutically acceptable salt thereof, which comprises reacting a compound of the formula IV:

IV
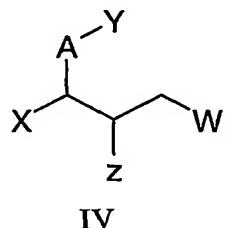
where A, X, Y and Z are as formula I above, and W is a leaving group, with an amine NR_1R_2 where R_1 and R_2 are as formula I above, optionally followed by the step of forming a pharmaceutically acceptable salt. Examples of suitable leaving groups include halo, mesylate and tosylate, but the nature of the leaving group is not critical. The reaction may be carried out in a sealed vessel with a lower alkyl alcohol as solvent.

The present invention also provides a process for producing a compound of formula I above wherein R_2 is H, or a pharmaceutically acceptable salt thereof, which comprises deprotecting a compound of the formula V:

V
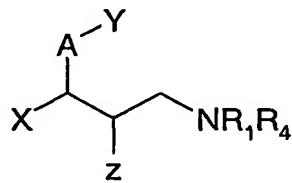
where A, X, Y, Z and R_1 are as formula I above, and R_4 is a suitable N-protecting group, optionally followed by the step of forming a pharmaceutically acceptable salt. Examples

-14-

of suitable N-protecting groups will be known to the person skilled in the art and include, for example, benzyl and t-butoxycarbonyl.

The present invention also provides a process for producing a compound of formula I above wherein Z is OH, or a pharmaceutically acceptable salt thereof, which comprises reacting a compound of the formula VI:

$$
\begin{array}{c}
A\text{-}Y \\
X \diagdown \text{CH} \diagdown \triangle_O
\end{array}
$$

VI
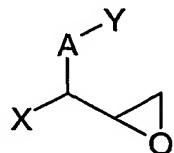
where A, X and Y are as formula I above with an amine NR_1R_2 where R_1 and R_2 are as formula I above, optionally followed by the step of forming a pharmaceutically acceptable salt.

The present invention also provides a process for producing a compound of formula I above wherein R_1 and R_2 are H, or a pharmaceutically acceptable salt thereof, which comprises reducing a compound of the formula VII:
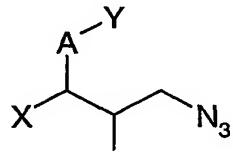
$$
\begin{array}{c}
A\text{-}Y \\
X \diagdown \text{CH} \diagdown \underset{Z}{\text{CH}} \diagdown N_3
\end{array}
$$

VII

where A, X, Y and Z are as formula I above, optionally followed by the step of forming a pharmaceutically acceptable salt. Examples of suitable reducing agents will be known to the person skilled in the art.

The present invention also provides a process for producing a compound of formula I above wherein R_1 and R_2 are C_1-C_4 alkyl, or a pharmaceutically acceptable salt thereof,

which comprises N-protecting a compound of the formula VIII by the introduction of two C_1–C_4 alkyl groups:

$$X \overset{\overset{\displaystyle A^{-Y}}{|}}{\underset{\underset{\displaystyle Z}{|}}{\diagdown}} NH_2$$

VIII
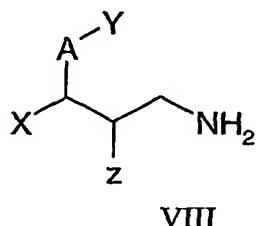
5

where A, X, Y and Z are as formula I above, optionally followed by the step of forming a pharmaceutically acceptable salt. Examples of suitable reagents for effecting N-protection by two C_1–C_4 alkyl groups will be known to the person skilled in the art.

10   Compounds of the present invention are inhibitors, preferably selective inhibitors, of the reuptake of both serotonin and norepinephrine and as such are useful as pharmaceuticals. They are particularly useful for the treatment of pain.

For clinical purposes, pain may be divided into two categories: acute pain and persistent
15   pain. Acute pain is provoked by noxious stimulation produced by injury and/or disease of skin, deep somatic structures or viscera, or abnormal function of muscle or viscera that does not produce actual tissue damage. On the other hand, persistent pain can be defined as pain that persists beyond the usual course of an acute disease or a reasonable time for an injury to heal or that is associated with a chronic pathologic process that causes
20   continuous pain or the pain recurs at intervals for months or years. If pain is still present after a cure should have been achieved, it is considered persistent pain. For the purpose of the present invention, persistent pain can be chronic non-remitting or recurrent. The difference in definition between acute and persistent pain is not merely semantic but has an important clinical relevance. For example, a simple fracture of the wrist usually
25   remains painful for a week to 10 days. If the pain is still present beyond the typical course of treatment, it is likely that the patient is developing reflex sympathetic dystrophy, a persistent pain syndrome that requires immediate effective therapy. Early·and effective

-16-

intervention potentially prevents the undue disability and suffering, and avoids the potential development of a condition that becomes refractory to therapy.

Acute and chronic pain differ in etiology, mechanisms, pathophysiology, symptomatology, diagnosis, therapy, and physiological responses. In contrast to the transitory nature of acute pain, persistent pain is caused by chronic pathologic processes in somatic structures or viscera, by prolonged and sometimes permanent dysfunction of the peripheral or central nervous system, or both. Also, persistent pain can sometimes be attributed to psychologic mechanisms and/or environmental factors.

Current therapies for persistent pain include opiates, barbiturate-like drugs such as thiopental sodium and surgical procedures such as neurectomy, rhizotomy, cordotomy, and cordectomy.

The compounds of the present invention are indicated in the treatment of persistant pain and references herein to pain are intended to refer to persistent pain.

In addition to the compounds of formula I and processes for the preparation of said compounds, the present invention further provides pharmaceutical compositions comprising a compound of formula I or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

Further, the present invention provides a compound of formula I or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical; and a compound of formula I or a pharmaceutically acceptable salt thereof, for use as a selective inhibitor of the reuptake of both serotonin and norepinephrine.

The present compounds and salts may be indicated in the treatment of disorders associated with serotonin and norepinephrine dysfunction in mammals.

The term "serotonin and norepinephrine dysfunction" as used herein refers to a reduction in the amount of serotonin and norepinephrine neurotransmitters within the synaptic cleft

-17-

below that which would be considered to be normal or desirable for a species, or an individual within that species. Thus the phrase "disorders associated with serotonin and norepinephrine dysfunction in mammals" refers to disorders which are associated with a reduction in the amount of serotonin and norepinephrine neurotransmitters within the synaptic cleft below that which would be considered to be normal or desirable for the mammalian species, or an individual within the species, in question. Some examples of disorders currently believed to be associated with reduced levels of serotonin and norepinephrine within the synaptic cleft include depression, OCD, anxiety, memory loss, urinary incontinence (including stress urinary incontinence and urge incontinence), conduct disorders, attention-deficit disorder (including ADHD), obesity, hot flushes/flashes, pain (including inflammatory pain, neuropathic pain, non-neuropathic non-inflammatory pain, persistent pain, persistent pain of inflammatory and/or neuropathic origin, headache and migraine), eating disorders (including bulimia and anorexia nervosa), inflammatory bowel disorders, functional bowel disorders, dyspepsia, chron's disease, iletis, ischemic bowel disease, ulcerative colitis, gastroesophageal reflux for functional bowel disorders, irritable bowel syndrome, insterstitial cystitis, urethral syndrome, gastric motility disorders, substance abuse (including alcoholism, tobacco abuse, smoking cessation, symptoms caused by withdrawal or partial withdrawal from the use of tobacco or nicotine and drug addiction including cocaine abuse), dementia of ageing, senile dementia, Alzheimer's, Parkinsonism, social phobia, disruptive behavior disorders, impulsive control disorders, borderline personality disorder, chronic fatigue syndrome, panic disorders, post-traumatic stress disorder, schizophrenia, gastrointestinal disorders, cardiovascular disorders, emesis, sleep disorders, cognitive disorders, psychotic disorders, brain trauma, premenstrual syndrome or late luteal syndrome, sexual dysfunction (including premature ejaculation and erectile difficulty), autism, mutism and trichotilomania. The compounds of the present invention are particularly suitable for the treatment of pain.

The compounds of the present invention are also indicated for the treatment of disorders which are ameliorated by an increase in the amount of serotonin and norepinephrine neurotransmitters within the synaptic cleft of a mammal above that which would be

-18-

considered to be normal or desirable for the mammalian species, or an individual within the species, in question.

The term "treatment" as used herein refers to both curative and prophylactic treatment of disorders associated with norepinephrine dysfunction.

The present invention also provides the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for selectively inhibiting the reuptake of serotonin and norepinephrine; the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of disorders associated with serotonin and norepinephrine dysfunction in mammals; the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disorder selected from those listed above and in particular selected from depression, OCD, anxiety, memory loss, urinary incontinence, conduct disorders, ADHD, obesity, alcoholism, smoking cessation, hot flushes/flashes and pain; and the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a disorder selected from depression, urinary incontinence, particularly stress induced urinary incontinence, and more especially, pain. The present invention further provides a compound of formula I for treating disorders associated with serotonin and norepinephrine dysfunction in mammals, for example a disorder selected from those listed above and in particular selected from depression, OCD, anxiety, memory loss, urinary incontinence, conduct disorders, ADHD, obesity, alcoholism, smoking cessation, hot flushes/flashes and pain, especially depression, urinary incontinence, particularly stress induced urinary incontinence, and, more especially, pain.

Further the present invention provides a method for selectively inhibiting the reuptake of serotonin and norepinephrine in mammals, comprising administering to a patient in need thereof an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof; a method for treating disorders associated with serotonin and norepinephrine dysfunction in mammals, comprising administering to a patient in need thereof an

-19-

effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof; and a method for treating a disorder selected from those listed above and in particular selected from depression, OCD, anxiety, memory loss, urinary incontinence, conduct disorders, ADHD, obesity, alcoholism, smoking cessation, hot flushes/flashes
5   and pain, comprising administering to a patient in need thereof an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

The present invention includes the pharmaceutically acceptable salts of the compounds of formula I. Suitable salts include acid addition salts, including salts formed with inorganic
10   acids, for example hydrochloric, hydrobromic, nitric, sulphuric or phosphoric acids, or with organic acids, such as organic carboxylic acids, for example pyruvic, lactobionic, glycolic, oxalic, maleic, hydroxymaleic, fumaric, malic, tartaric, citric, salicylic, o-acetoxybenzoic, or organic sulphonic, 2-hydroxyethane sulphonic, toluene-p-sulphonic, bisethanesulphonic acid or methanesulphonic acid.
15

In addition to the pharmaceutically acceptable salts, other salts are included in the invention. They may serve as intermediates in the purification of compounds or in the preparation of other, for example pharmaceutically acceptable, acid addition salts, or are useful for identification, characterisation or purification.
20

While all the compounds of the present invention are believed to inhibit the reuptake of serotonin and norepinephrine in mammals there are certain of these compounds which are preferred for such uses. Preferred values for A, X, Y, Z, R_1 and R_2 and substituents for each have been set out above.
25

Compounds of the present invention may be prepared by conventional organic chemistry techniques as described below.

When Z is H, 1-X-3-aminopropanols can be prepared via the corresponding 3-amino-N-
30   methoxy-N-methylpropanamide, known as a Weinreb amide, as follows:

-20-
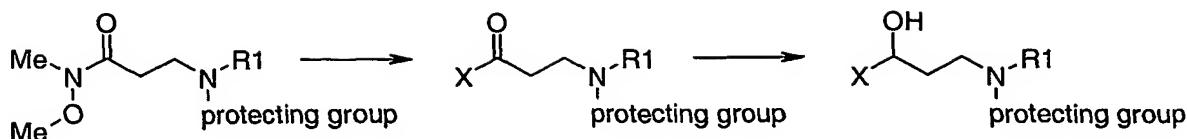
Subjecting a Weinreb amide of N-methyl β-alanine appropriately protected at the nitrogen, for example as a t-butyl carbamate (Boc) or as a benzyl amine, to an

5   organometallic reagent like an alkyl Grignard or alkyl lithium results in the desired X-substituted ketone. The ketone can be readily reduced to the desired racemic alcohol using standard reducing agents such as sodium borohydride in a protic solvent such as lower order alkyl alcohols.

10   The Weinreb amides of this invention may be prepared by conventional organic chemistry techniques as exemplified below:
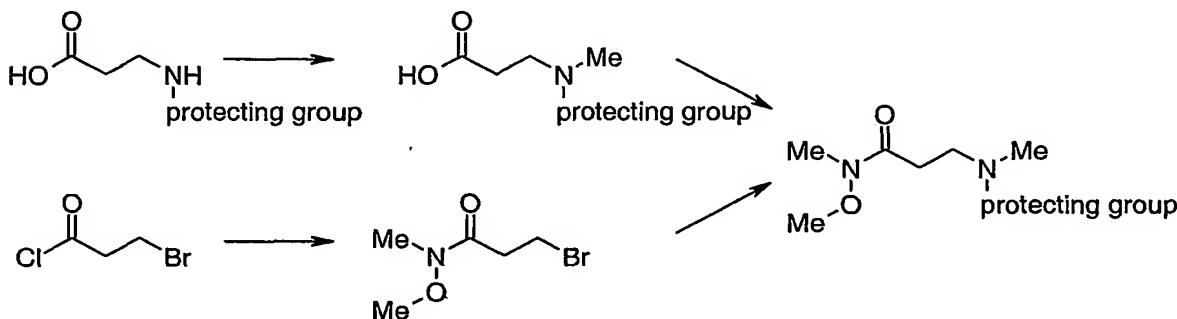
15   Subjecting a commercial available appropriately N-protected β-alanine to sodium hydride followed by methyl iodide results in the N-methylated derivative, which then can be converted to the Weinreb amide by reaction with N-methyl-O-methylhydroxylamine. The Weinreb amides can also be prepared by reacting a 3-bromopropanoyl chloride with N-methyl-O-methylhydroxylamine to give the Weinreb amide of 3-bromopropanoic acid,

20   which then can be substituted with an appropriately substituted amine to give the desired Weinreb amide.

When Z is H, another preferred route to 1-X-3-aminopropanols is addition of a suitable organometallic reagent to an appropriately N-protected aminoaldehyde. Thus an

25   appropriately protected amine can be added to a vinyl aldehyde in a Michael addition

-21-

reaction to give a 3-aminopropanal. This aminoaldehyde can be subjected to (for example) an alkyl Grignard reagent or an alkyl lithium reagent to give the desired 1-alkylpropanol. Selection of other Grignard reagents or organolithium reagents could provide other 1-X-3-aminopropanols.

5
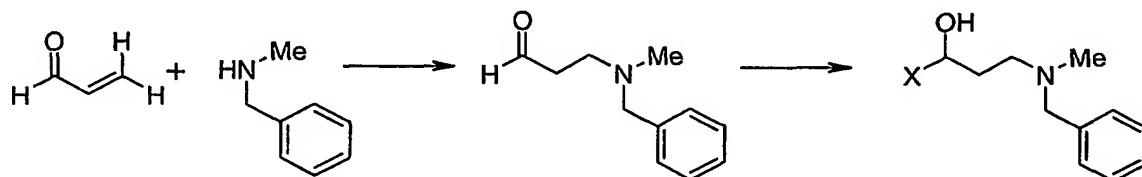
The benzothiophenes of the invention have been made by several routes. Thus a preferred route is by the alkylation of a thiophenol derivative with bromoacetaldehyde diethyl acetal

10   followed by subsequent acid catalysed cyclisation with polyphosphoric acid in chlorobenzene with elimination of ethanol. Subsequent demethylation provided the hydroxybenzothiophene needed for subsequent ether formation.

15

Analogous cyclisation of phenylthioacetone derivatives with PPA can be used to synthesise 3-methyl derivatives of benzothiophene methyl ethers.
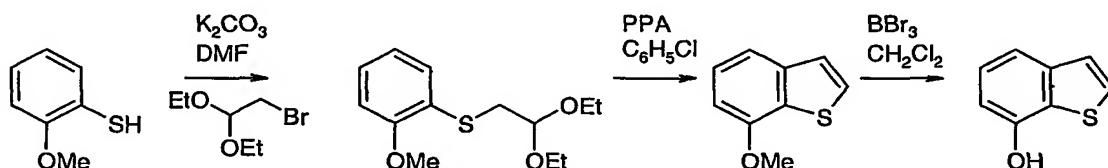
A further preferred route to substituted benzothiophenes is an iodine catalysed cyclisation

20   of a mercaptopropenoic acid. Thus a benzaldehyde can be condensed with rhodanine and subsequently hydrolysed under basic conditions to a mercaptopropenoic acid. The resultant mercaptopropenoic acid can be cyclised using iodine and then decarboxylated with diazobicycloundecane in dimethylacetamide. Finally boron tribromide in dichloromethane may be used to demethylate the methyl ether to provide the

25   hydroxybenzothiophene.

-22-
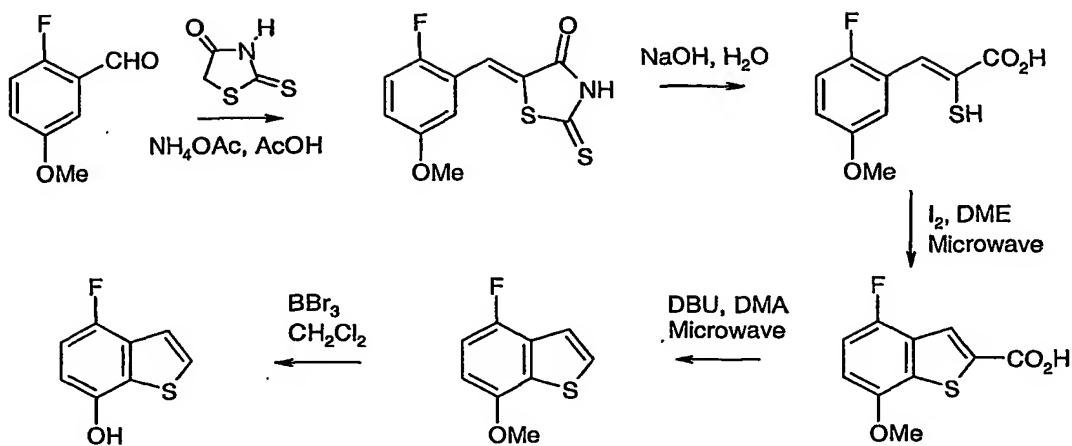
Methods for the synthesis of other Y-OH precursors of use in the present invention (or literature references describing their synthesis) are provided in the experimental section

5   below.

The corresponding ethers and thioethers can generally be prepared as follows:
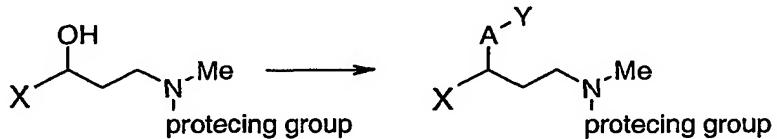
10

The propanols can be subjected to O-arylation or S-arylation reactions. Various O-arylation conditions can be used such as the Mitsunobu reaction, wherein roughly equal quantities of the heteroaryl alcohol and the 1-X,3-aminopropanol are stirred at temperatures of between 0 °C and reflux in a polar non protic solvent such as toluene,

15   with a complexing agent such as 1,1'(azodicarbonyl)dipiperidine, or another derivative, and a phosphine ligand such as tributylphosphine. This type of reaction is well known and further combinations of the Mitsunobu reagents can be found in Organic Preparations and Procedures Int., 1996, 28, 2, 165 and references therein. For converting hydroxy to aryl sulfide it is preferred to react the propanol species with Y-SH,

20   (cyanomethyl)trimethylphosphonium iodide (Tetrahedron, 2001, 57, 5451-5454) and diisopropylamine in propionitrile.
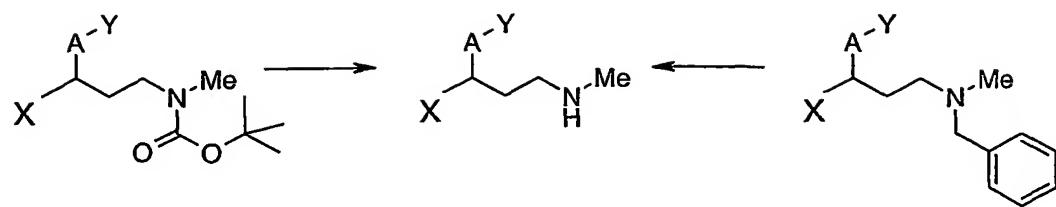
The corresponding protected amines can be readily converted to the corresponding amines by standard methods. For example, trifluoroacetic acid can be used for deprotection of a

5   *N-t*-butyloxycarbonyl group and 1-chloroethyl chloroformate in dichloroethane followed by methanol can be used to remove the benzyl group. Finally, resolution of the obtained racemates can be obtained by chiral chromatography.

The "anti" chain hydroxylated propanamines may be prepared using the methodology

10   outlined below.
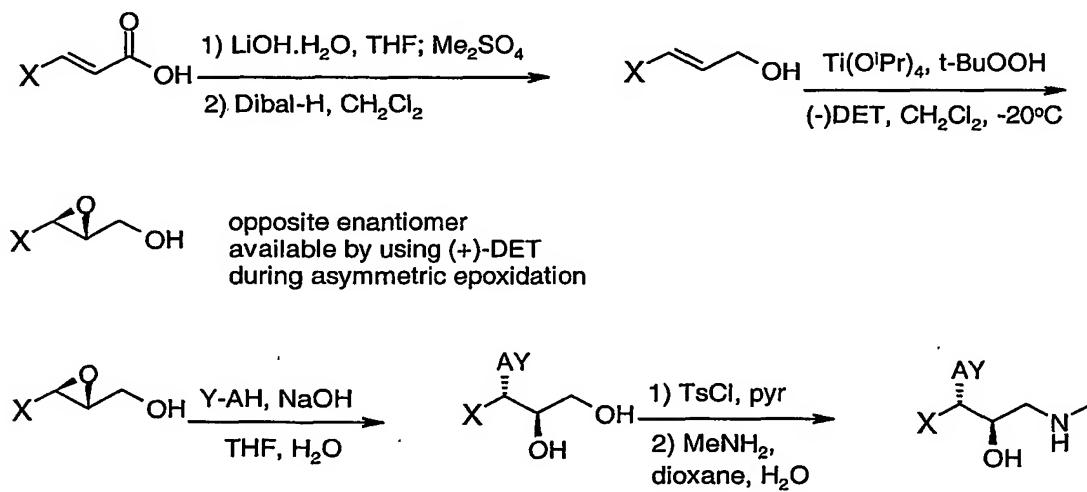
The "syn" chain hydroxylated propanamines may be prepared using the methodology

15   outlined below.

-24-

(I)

(II)
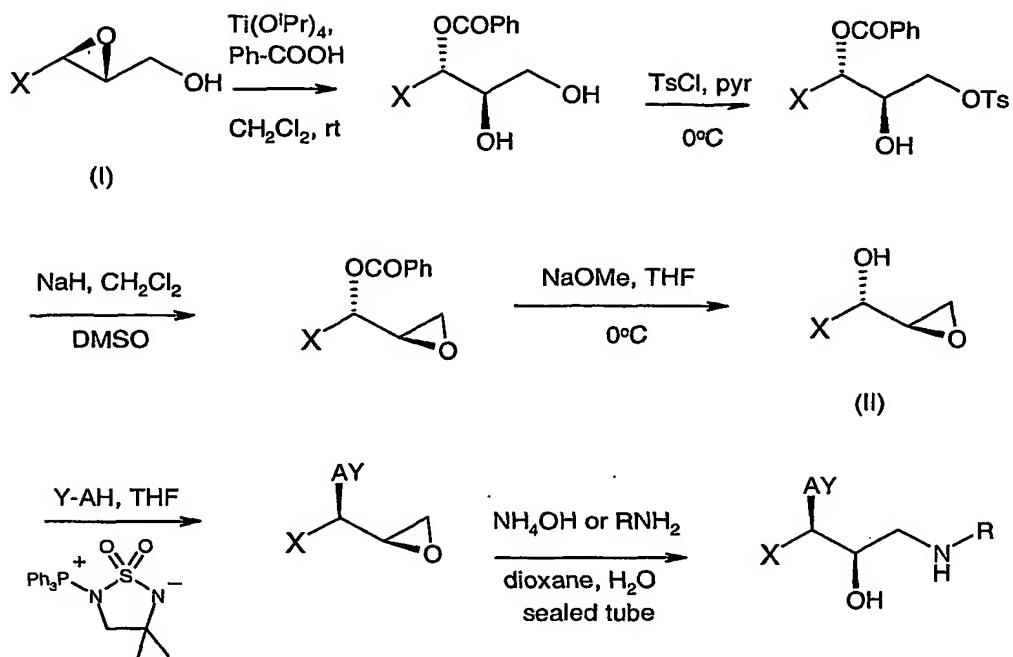
Alternatively, the "syn" chain hydroxylated propanamines may be prepared using the method outlined below.

5

(I)

(II)
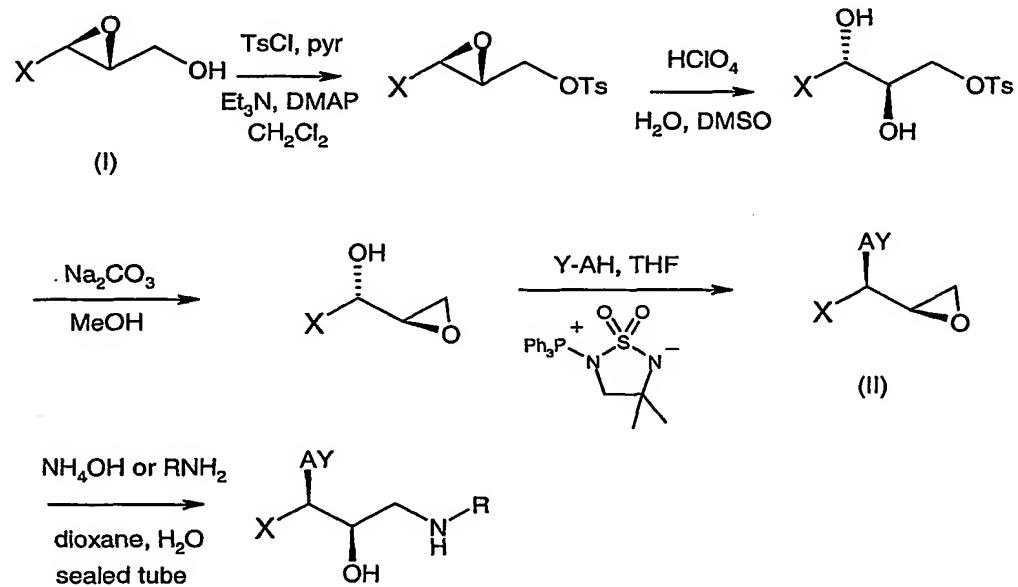
Alternatively, the "syn" and "anti" chain hydroxylated propanamines may be prepared using the intermediates shown below.

-25-
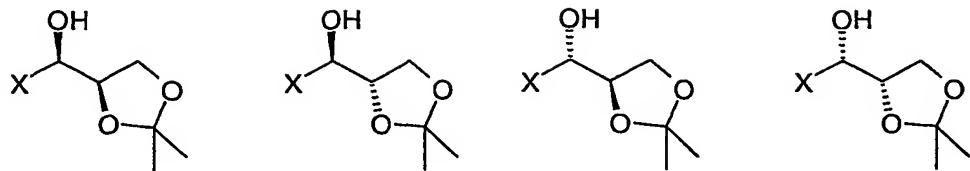
The same chemical transformations may be applied to each intermediate to obtain each of the four stereochemically distinct final products. For example:

5
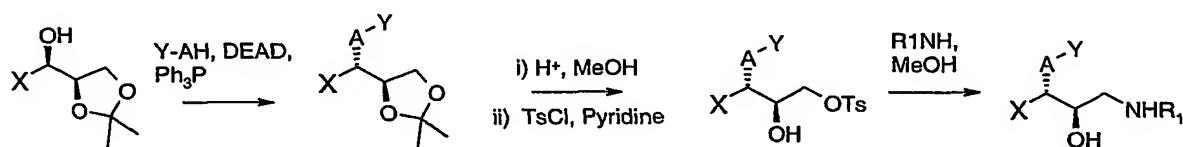
Starting from the other three intermediates, the same chemistry may be used to obtain the other three final products, i.e.

10
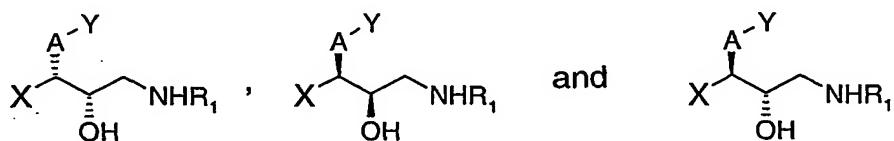
The four intermediates may be synthesized by two different routes. The first route is shown below:

15
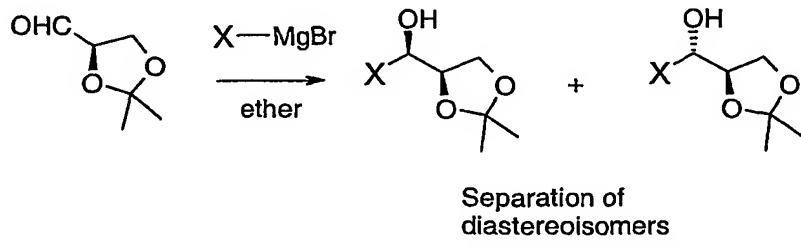
Separation of
diastereoisomers

The other two intermediates may be obtained using the same chemistry but starting with the compound:
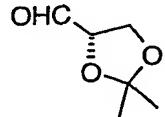
-26-

The second route is shown below:
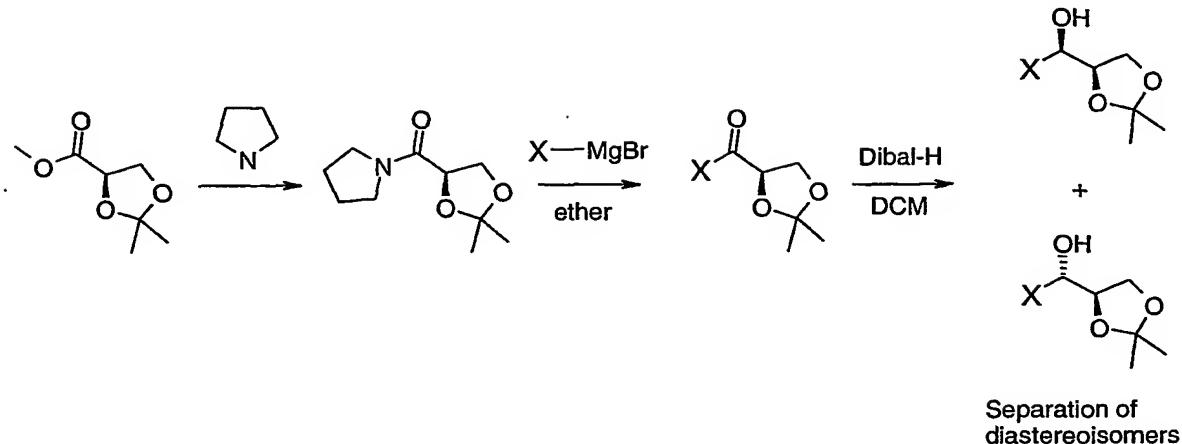
Separation of
diastereoisomers

The other two intermediates may be obtained using the same chemistry but starting with
the compound:
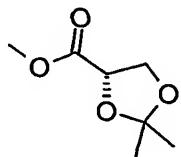
5

The preparation of the reagent 4,4-(dimethyl-1,1-dioxido-1,2,5-thiadiazolidin-2-yl)-
triphenyl phosphonium is described in J. Org. Chem. 1994, 59, 2289. Note however that
for converting hydroxy to aryl sulfide it is preferred to react the propanol species with Y-
10   SH, (cyanomethyl)trimethylphosphonium iodide (Tetrahedron, 2001, 57, 5451-5454) and
diisopropylamine in propionitrile.

The "syn" chain fluorinated propanamines may be prepared using the method outlined
below.
15

-27-
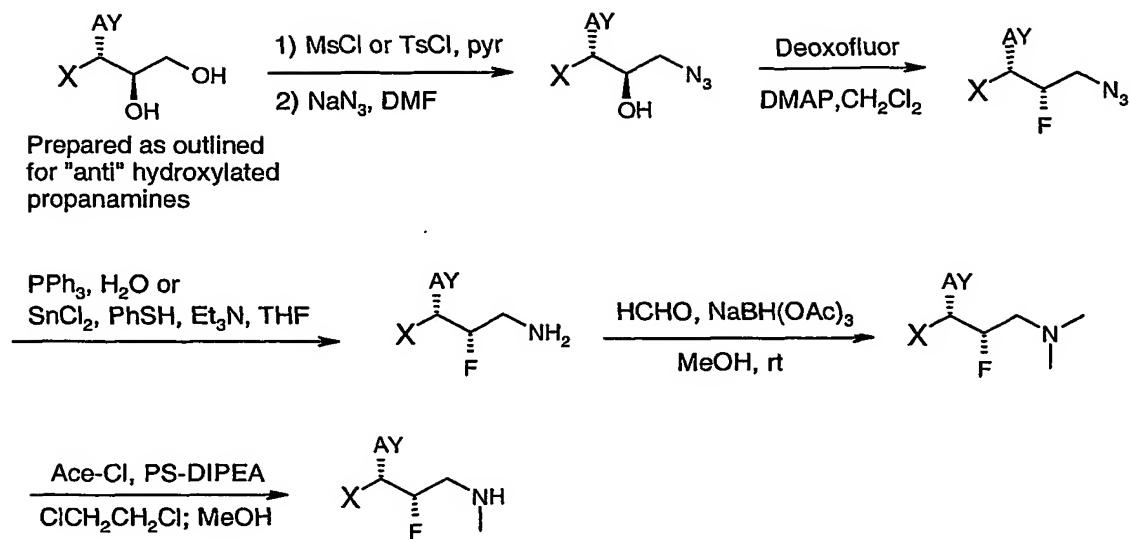
The "anti" chain fluorinated propanamines may be prepared using the method outlined below.

5
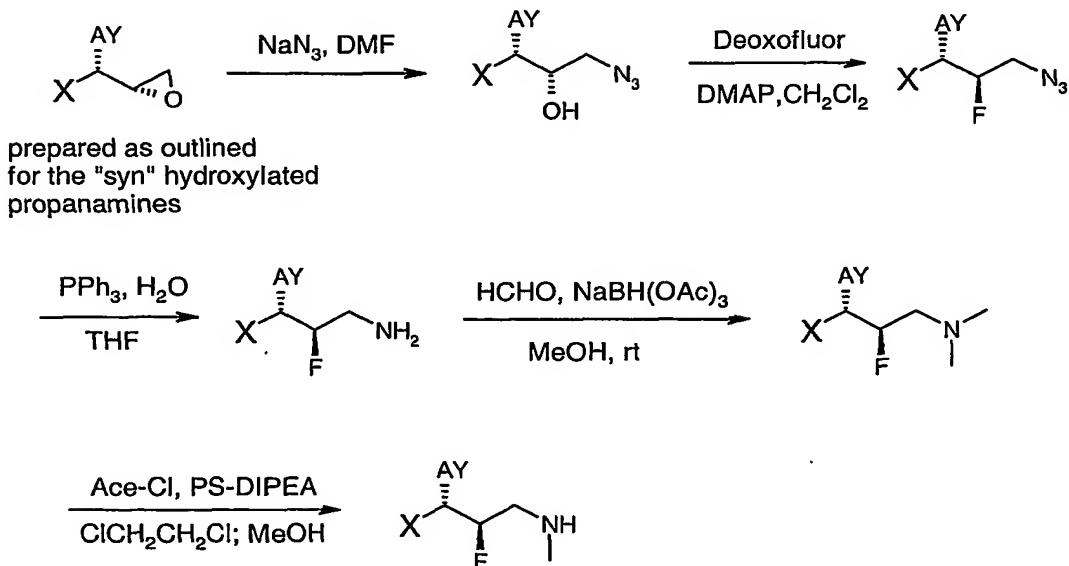
The following Examples further illustrate the compounds of the present invention and methods for their synthesis.

10

In the following section, there is described the synthesis of precursors and common intermediates for the compounds of the present invention.

-28-

4-Fluoro-2-methoxybenzenethiol

a) 2-Bromo-5-fluoroanisole
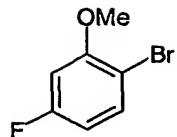
5   To a suspension of 2-bromo-5-fluorophenol (20.0 g, 104.7 mmol) and potassium carbonate (21.71 g, 157.1 mmol) in acetone (200 mL) was added dimethyl sulphate (10.90 mL, 115.2 mmol). The resulting suspension was allowed to stir at 60°C for 2 h before being allowed to cool and then concentrated *in vacuo*. The residue was dissolved in ether (200 mL) and water (100 mL). The organic phase was washed with aqueous hydrochloric

10   acid (2 N, 50 mL), saturated sodium bicarbonate solution (50 mL) with the resulting organic phase being dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give a pale yellow oil (21.46 g, 100%). δ_H (300 MHz, CDCl$_3$) 7.45 (1H, dd, Ar), 6.70-6.55 (2H, m, Ar), 3.90 (3H, s, OCH_3).


15   b) 4-Fluoro-2-methoxybenzenethiol
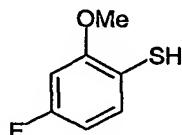
To a suspension of 2-bromo-5-fluoroanisole (2.00 g, 9.755 mmol) and elemental sulphur (0.468 g, 14.632 mmol) in dry THF (50 mL) was slowly added *tert*-butyl lithium in pentane (1.7 M, 12.6 mL, 21.46 mmol) at –78°C. The resulting suspension was allowed to

20   stir at -78°C for 60 mins before being poured onto saturated ammonium chloride solution (80 mL) and product extracted with diethyl ether (100 mL). The organic phase was washed with aqueous hydrochloric acid (2 N, 40 mL), with the resulting organic phase being dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give a pale yellow oil. This was treated to a pad of silica gel, eluting with hexane:ethyl acetate [95:5] to give a pale

25   yellow oil (1.50 g, 68%). δ_H (300 MHz, CDCl$_3$) 7.20 (1H, dd, Ar), 6.65-6.55 (2H, d, Ar), 3.90 (3H, s, OCH_3), 3.68 (1H, s, SH).

-29-

Similarly prepared was

3-Methoxy-5-trifluoromethylbenzenethiol as a pale yellow oil (14.473g, 100%). δ_H (300 MHz, CDCl$_3$) 7.40-6.90 (3H, m, Ar), 3.87 (1H, s, SH), 3.80 (3H, s, OCH_3).

3-Fluoro-2-methoxybenzaldehyde
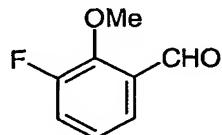
To a suspension of 3-fluoro-2-hydroxybenzaldehyde (5.328 g, 38.02 mmol) and potassium carbonate (7.88 g, 57.03 mmol) in acetone (60 mL) was added dimethyl sulphate (3.96 mL, 41.83 mmol). The resulting suspension was stirred at 60°C for 2 h before being allowed to cool and then concentrated *in vacuo*. The residue was dissolved in dichloromethane (100 mL) and water (50 mL). The organic phase was washed with saturated sodium bicarbonate (50 mL) with the resulting organic phase being dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give a pale yellow oil (6.262 g, 38.02 mmol, 100%). δ_H (300 MHz, CDCl$_3$) 10.40 (1H, s, CHO), 7.60 (1H, d, Ar), 7.30 (1H, m, Ar), 7.10 (1H, m, Ar), 4.10 (3H, s, OCH_3).

5-Methoxy-2-methylbenzaldehyde

a) 4-Bromo-3-(1,3-dioxolan-2-yl)phenyl methyl ether

A solution of 2-bromo-5-methoxybenzaldehyde (10.00 g , 46.5 mmol) in toluene (600 mL), ethanediol (3.88 mL, 69.8 mmol) and *para*-toluene sulphonic acid (50 mg) were heated under Dean-Stark conditions for 24 h. After this time the reaction was allowed to cool to room temperature before being washed with saturated aqueous sodium hydrogen carbonate (2 x 150 mL). The organic phase was dried (MgSO$_4$) and the solvent removed *in vacuo* to give a colourless oil (12.6 g, 100%); R$_f$ = 0.23 in hexane:ethyl acetate [10:1];

-30-

δ_H (300 MHz, CDCl$_3$) 7.42 (1H, d, Ar), 7.25 (1H, d, Ar), 6.75 (1H, dd, Ar), 6.03 (1H, s, C*H*O), 4.20-4.01 (4H, m, 2 x C*H*$_2$), 3.80 (3H, s, OC*H*$_3$).

b) 3-(1,3-Dioxolan-2-yl)-4-methylphenyl methyl ether
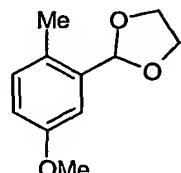
5

n-Butyl lithium in hexane (17.07 mL, 1.42 M, 25.1 mmol) was added dropwise at -78°C to a stirred solution of 4-bromo-3-(1,3-dioxolan-2-yl)phenyl methyl ether (5.00 g, 19.3 mmol) in dry THF (60 mL). The resulting solution was allowed to stir at -78°C for 30 mins before being quenched with iodomethane (2.40 mL, 38.6 mmol). The resulting solution was stirred at -78°C for a further 20 mins before being quenched with saturated aqueous ammonium chloride solution (60 mL). The organic phase was dried (MgSO$_4$) and the solvent evaporated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [10:1] to give a pale yellow oil (3.07 g, 82%); R$_f$ = 0.40 in hexane:ethyl acetate [10:1]; δ_H (300 MHz, CDCl$_3$) 7.20-6.99 (2H, m, Ar), 6.75 (1H, dd, Ar), 5.92 (1H, s, C*H*), 4.20-4.01 (4H, m, 2 x C*H*$_2$), 3.80 (3H, s, OC*H*$_3$), 2.32 (3H, s, C*H*$_3$).

c) 5-Methoxy-2-methylbenzaldehyde
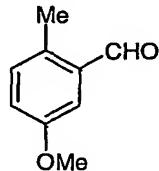
A solution of 2-(2-methyl-5-methoxyphenyl)-1,3-dioxalone (7.28 g, 37.5 mmol) in THF (1200 mL) and HCl (5%, 50 mL) was stirred at room temperature for 48 h. After this time the reaction was diluted with diethyl ether (100 mL) and washed with brine. The organic phase was dried (MgSO$_4$) and the solvent evaporated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [95:5] to give a pale yellow oil (4.93 g, 88%); R$_f$ = 0.31 in hexane:ethyl acetate [10:1]; δ_H (300 MHz, CDCl$_3$) 10.28 (1H, s, C*H*O), 7.35-6.99 (3H, m, Ar), 3.82 (3H, s, OC*H*$_3$), 2.62 (3H, s, C*H*$_3$).

1-[(5-Fluoro-2-methoxyphenyl)thio]-2-propanone
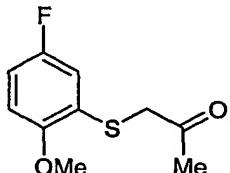
tert-Butyl lithium in pentane (6.30 mL, 10.7 mmol) was added dropwise at -78°C over 35
mins to a stirred suspension of 2-bromo-4-fluoroanisole (1.00 g, 4.87 mmol) and
elemental sulfur (234 mg, 7.31 mmol) in dry THF (10 mL). The resulting yellow solution
was stirred at -78°C for 15 mins before 1-chloro-2-propanone (894 mg, 9.74 mmol) was
added. The resulting solution was allowed to stir at -78°C for 1 hr before being quenched
with NH$_4$Cl (sat., 20 mL). The organic phase was extracted and dried (MgSO$_4$) and the
solvent removed *in vacuo*. The residue was purified by flash chromatography eluting
silica gel with hexane:ethyl acetate [4:1] to yield a colourless oil (1.03 g, 98%) which
slowly solidified on standing; R_f = 0.34 in hexane:ethyl acetate [4:1]; δ_H (300 MHz,
CDCl$_3$) 7.08-6.98 (1H, dd, Ar), 6.95-6.82 (1H, m, Ar), 6.80-6.71 (1H, dd, Ar), 3.91 (3H,
s, OCH_3), 3.75 (2H, s, CH_2), 2.30 (3H, s, CH_3).

1-[(2-Fluoro-5-methoxyphenyl)thio]-2-propanone
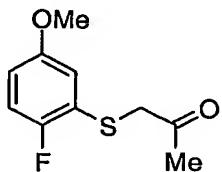
To a solution of 2,2,6,6-tetramethylpiperidine (8.03 mL, 47.6 mmol) in THF (20 mL) at -
78°C was added a solution of 2.5 M *n*-butyllithium in hexanes (19.04 mL, 47.6 mmol).
After stirring for 30 minutes at -78°C a solution of 4-fluoroanisole (5 g, 39.7 mmol) in
THF (10 mL) was added dropwise. After a further 30 minutes elemental sulphur (1.78 g,
55.5 mmol) was added and stirred until almost all of the sulphur has disappeared. 1-
chloro-2-propanone (3.79 mL, 47.6 mmol) was then added and the solution warmed to
room temperature over 2 hours. The reaction was quenched by pouring into saturated
ammonium chloride (50 mL) and extraction with diethyl ether (2 x 50 mL). The
combined organic extracts were dried (MgSO$_4$) and the solvent removed *in vacuo* to give

-32-

a brown oil which was purified by flash chromatography with a gradient of 0-5% diethyl ether in hexane to give the title compound (1.96 g, 23%); δ_H (300 MHz, CDCl$_3$) 7.02-6.86 (2H, m, ArH), 6.80-6.71 (1H, m, ArH), 3.78 (3H, s, OCH$_3$), 3.68 (2H, s, CH$_2$) and 2.29 (3H, s, CH$_3$).

1-Benzothien-7-yl methyl ether

a) 1-[(2,2-Diethoxyethyl)thio]-2-methoxybenzene
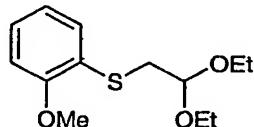
To a suspension of 2-methoxybenzenethiol (10 g, 71.4 mmol) and potassium carbonate (20 g, 143 mmol) in dry N,N-dimethylformamide (100 mL) was added dropwise over 20 mins a solution of bromoacetaldehyde diethyl acetal (10.3 mL, 71.4 mmol) in dry N,N-dimethylformamide (50 mL). The resulting suspension was allowed to stir at room temperature for 45 mins before being diluted with water (500 mL) and extracted with hexane (200 mL). The organic phase was further extracted with brine (4 x 100 mL), with the resulting organic phase being dried (MgSO$_4$) and the solvent removed *in vacuo* to give a pale yellow oil (18.6 g) which was ca 95% pure. This material was further purified by flash chromatography eluting silica gel with hexane:ether [10:1] to give a colourless oil (18 g, 98%). δ_H (300 MHz, CDCl$_3$) 7.34 (1H, d, Ar), 7.2 (1H, m, Ar), 6.92-6.80 (2H, m, Ar), 4.62 (1H, t, J = 7 Hz, CH(OEt)$_2$), 3.85 (3H, s, OCH_3), 3.70-3.42 (4H, m, OCH_2CH$_3$), 3.10 (2H, d, J = 7 Hz, SCH_2), 1.12 (6H, t, J = 7 Hz, OCH$_2$CH_3).

Similarly prepared were

1-[(2,2-Diethoxyethyl)thio]-4-fluoro-2-methoxybenzene as a colourless oil (0.828 g, 3.018 mmol, 46%). δ_H (300 MHz, CDCl$_3$) 7.38 (1H, dd, Ar), 6.90 (2H, m, Ar), 4.62 (1H, t, J = 7 Hz, CH(OEt)$_2$), 3.85 (3H, s, OCH_3), 3.70-3.45 (4H, m, OCH_2CH$_3$), 3.00 (2H, d, J = 7 Hz, SCH_2), 1.18 (6H, t, J = 7 Hz, OCH$_2$CH_3).

1-[(2,2-Diethoxyethyl)thio]-3-methoxy-5-trifluoromethylbenzene as a pale orange oil (6.87 g, 21.18 mmol, 34%). δ_H (300 MHz, CDCl$_3$) 7.20 (1H, s, Ar), 7.00 (1H, s, Ar), 6.90

-33-

· (1H, s, Ar), 4.65 (1H, t, J = 7 Hz, CH(OEt)$_2$), 3.80 (3H, s, OCH_3), 3.70-3.50 (4H, m, OCH_2CH$_3$), 3.15 (2H, d, J = 7 Hz, SCH_2), 1.20 (6H, t, J = 7 Hz, OCH$_2$CH_3).

b) 1-Benzothien-7-yl methyl ether
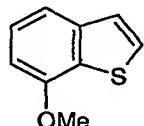
A solution of 1-[(2,2-diethoxyethyl)thio]-2-methoxybenzene (18 g, 70.3 mmol) in dry chlorobenzene (100 mL) was added slowly to a stirred solution of polyphosphoric acid (50g) in dry chlorobenzene (300 mL) at 145°C. After addition was complete the resulting black solution was stirred at 155°C for a further 18 hrs. After this time the reaction was allowed to cool to room temperature before being filtered through a pad of CELITE™, the solid cake was washed with dichloromethane (200 mL) and the combined organic extracts were concentrated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [95:5] to give a pale yellow oil (8.7 g, 75%); δ_H (300 MHz, CDCl$_3$) 7.45-7.29 (4H, m, Ar), 6.75 (1H, d, Ar), 4.00 (3H, s, OCH_3).


Similarly prepared were

5-Fluoro-1-benzothien-7-yl methyl ether as a pale yellow oil (0.132 g, 0.724 mmol, 24%); δ_H (300 MHz, CDCl$_3$) 7.50 (1H, d, Ar), 7.28 (1H, d, Ar), 7.10 (1H, d, Ar), 6.58 (1H, d, Ar), 4.00 (3H, s, OCH_3).

4-Trifluoromethyl-1-benzothien-6-yl methyl ether as a yellow oil (2.832 g,12.19 mmol, 58%); δ_H (300 MHz, CDCl$_3$) 7.50-7.35 (3H, m, Ar), 7.28 (1H, s, Ar), 3.90 (3H, s, OCH_3).

4-Fluoro-7-methoxy-1-benzothiophene

a) 5-(2-Fluoro-5-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one
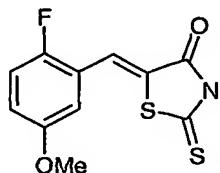
-34-

To a suspension of 2-fluoro-5-methoxybenzaldehyde (5.00 g, 32.46 mmol) and rhodanine (4.31 g, 32.46 mmol) in dry toluene (1000 mL) was added ammonium acetate (50 mg) and acetic acid (2 mL). The resulting suspension was allowed to stir at 120°C for 12 h under Dean-Stark apparatus before being allowed to cool and filtered. Resultant solid was
5   washed with hexane and allowed to dry *in vacuo* to give an orange crystalline solid (8.00 g, 91%); δ_H (300 MHz, CDCl$_3$) 7.50 (1H, s, CH=C); 7.31 (1H, t, Ar), 7.20-7.11 (1H, m, Ar), 6.95-6.89 (1H, m, Ar), 3.80 (3H, s, OCH$_3$).

Similarly prepared were

10

5-(3-Fluoro-2-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one as an orange solid (7.942 g, 78%). δ_H (300 MHz, CDCl$_3$) 7.82 (1H, s, ArCHCR$_2$), 7.30-7.10 (3H, m, Ar), 4.05 (3H, s, OCH$_3$).

15   5-(2-Methyl-5-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one   as   an   orange crystalline solid (8.00 g, 91%); δ_H (300 MHz, CDCl$_3$) 7.80 (1H, s, Ar), 7.29-7.10 (1H, m, Ar), 6.95-6.84 (2H, m, Ar and CH=C), 3.83 (3H, s, OCH$_3$), 2.37 (3H, s, CH$_3$).

b) (2Z)-3-(2-Fluoro-5-methoxyphenyl)-2-mercapto-2-propenoic acid

20

5-(2-Fluoro-5-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one   (8.00g,   9.7   mmol) was added in one portion to 25% w/v sodium hydroxide solution (40 mL). This was allowed stir at reflux for 1 h. After this time the reaction was allowed to cool to room temperature and poured onto water (50 mL). This was washed with dichloromethane (50
25   mL), and the aqueous layer acidified to pH 2 with aqueous hydrochloric acid (2 N, 50 mL) to give a white suspension. Product was extracted with ether (2 x 60 mL), dried (MgSO$_4$) and solvent removed *in vacuo* to give a white solid (6.71 g, 100%); δ_H (300 MHz, CD$_3$OD) 7.85 (1H, s, Ar), 7.46-7.35 (1H, m, Ar), 7.11 (1H, t, Ar), 7.01-6.75 (2H, m, CH=, and SH), 3.80 (3H, s, OCH$_3$).

Similarly prepared were

(2Z)-3-(3-Fluoro-2-methoxyphenyl)-2-mercapto-2-propenoic acid as a solid (1.596 g,

94%); δ_H (300 MHz, CDCl$_3$) 8.12 (1H, s, ArCHCR$_2$), 7.60 (1H, d, Ar), 7.15-7.00 (2H, m, Ar), 4.55 (1H, s, SH), 3.98 (3H, s, OCH_3).

(2Z)-3-(2-Methyl-5-methoxyphenyl)-2-mercapto-2-propenoic acid as a white solid (6.71 g 100%); δ_H (300 MHz, CDCl$_3$) 8.00 (1H, s, CH=C), 7.30-7.09 (2H, m, Ar), 6.88-6.78 (1H, m, Ar), 3.80 (3H, s, OCH_3), 2.25 (3H, s, CH_3).

c) 4-Fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid

(2Z)-3-(2-Fluoro-5-methoxyphenyl)-2-mercapto-2-propenoic acid (1.00 g, 4.38 mmol) was added in one portion to a solution of iodine (1.66 g, 6.56 mmol) in dimethoxyethane (10 mL). This was heated in the microwave with 300W at 160°C for 10 mins. After this time the reaction was allowed to cool to room temperature and poured onto saturated sodium metabisulphite (200 mL) and ether (400 mL). Ether layer was separated and product extracted with aqueous sodium hydroxide (2 N, 2 x 100 mL). This was then acidified to pH 2 with aqueous hydrochloric acid (2 N, 250 mL), and product extracted with ether (2 x 150 mL). The combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo* to give a white solid (580 mg, 30%); δ_H (300 MHz, CD$_3$OD) 8.00 (1H, s, Ar), 7.30-7.19 (1H, m, Ar), 7.10-7.00 (1H, m, Ar), 3.95 (3H, s, OCH_3).

Similarly prepared was

4-Methyl-7-methoxy-1-benzothiophene-2-carboxylic acid as white solid (580 mg, 30%); δ_H (300 MHz, CDCl$_3$) 8.20 (1H, s, Ar), 7.12 (1H, d, Ar), 6.75 (1H, d, Ar), 3.99 (3H, s, OCH_3), 2.59 (3H, s, CH_3).

d) 4-Fluoro-7-methoxy-1-benzothiophene
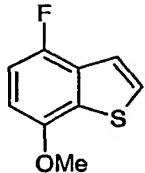
4-Fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid (2.00 g, 8.84 mmol) was added

5   in one portion to DBU (8 mL) and dimethyl acetamide (10 mL). This was heated in the
microwave with 300W at 200°C for 1 h. The reaction mixture was allowed to cool and
poured onto water (100 mL). Product was extracted with hexane (2 x 100 mL), washed
with aqueous hydrochloric acid (2 N, 50 mL), aqueous sodium hydroxide (2 N, 50 mL),
and the combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo*. The

10   residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate
[96:4] to give an oil (1.12 g, 70%); δ_H (300 MHz, CDCl$_3$) 7.4 (2H, s, Ar), 6.9 (1H, t, Ar),
6.60 (1H, dd, Ar), 3.91 (3H, s, OCH_3).

Similarly prepared was

15

4-Methyl-7-methoxy-1-benzothiophene as an oil (1.12 g, 70%); δ_H (300 MHz, CDCl$_3$)
7.46-7.32 (2H, m, Ar), 7.10 (1H, d, Ar), 6.66 (1H, d, Ar), 3.98 (3H, s, OCH_3), 2.52 (3H, s,
CH_3).

20   **5-Fluoro-4-methoxy-1-benzothiophene**

(2Z)-3-(3-Fluoro-2-methoxyphenyl)-2-mercapto-2-propenoic acid (4.865 g, 21.315 mmol)
was added in one portion to a solution of iodine (8.255 g, 31.973 mmol) in
dimethoxyetheane (30 mL). This was heated in the microwave with 300W at 120°C for 25

25   mins. After this time the reaction was allowed to cool to room temperature and poured
onto saturated sodium metabisulphite (200 mL) and ether (400 mL). Ether layer was
separated and product extracted with aqueous sodium hydroxide (2 N, 2 x 100 mL). This
was then acidified to pH 2 with aqueous hydrochloric acid (2 N, 250 mL), and product
extracted with ether (2 x 150 mL). The combined organic extracts were dried (MgSO$_4$)

-37-

and concentrated *in vacuo* to give a tan coloured solid (3.240 g, 14.322 mmol, 67%). Which was used without further purification in the next step. 5-Fluoro-4-methoxy-1-benzothiophene-2-carboxylic acid (0.883 g, 3.903 mmol) was added in one portion to DBU (2.04 mL, 13.661 mmol) and dimethyl acetamide (10 mL). This was heated in the microwave with 300W at 200 °C for 1 h. Reaction was allowed to cool and poured onto water (100 mL). Product was extracted with hexane (2x100 mL), washed with aqueous hydrochloric acid (2 N, 50 mL), aqueous sodium hydroxide (2 N, 50 mL), and the combined organic extracts were dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [96:4] to give a pale yellow oil (0.167 g, 23%); δ_H (300 MHz, CDCl$_3$) 7.60-6.80 (4H, m, Ar), 4.10 (3H, s, OCH_3).

7-Fluoro-4-methoxy-1-benzothiophene

a) 2-(5-Fluoro-2-methoxyphenyl)-2-hydroxy-*N,N*-dimethylethanethioamide
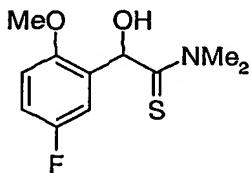
To a solution of lithium diisopropylamide, 2M in THF/n-heptane (210 mL, 583 mmol) was added THF (100 mL) and the solution cooled to -78°C under nitrogen. This was then added dropwise over 1 h to a solution of the 5-fluoro-2-methoxybenzaldehyde (50 g, 0.32 mmol) and *N,N*-dimethylthioformamide (34.7 g, 389 mmol) in dry THF (200 mL). This was warmed to -5°C and quenched with water (400 mL). The solution was filtered and washed with diethyl ether, the aqueous layer was extracted with ether (1 L). The combined organic layers were washed with water (500 mL), dried (MgSO$_4$) and the solvent removed *in vacuo* to give a crystalline suspension in oil. This was triturated in ether and filtered to give a crystalline solid (21.8 g, 28%); δ_H (300 MHz, CDCl$_3$) 7.16-6.81 (3H, m, ArH), 5.81-5.75 (1H, m, CHOH), 5.31-5.22 (1H, m, OH), 4.89 (3H, s, OCH$_3$), 3.50 (3H, s, N(CH$_3$)$_2$) and 3.08 (3H, s, N(CH$_3$)$_2$).

-38-

b) *N*-(7-Fluoro-4-methoxy-1-benzothien-2-yl)-*N,N*-dimethylamine
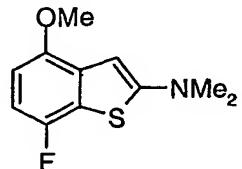
A solution of 2-(5-fluoro-2-methoxyphenyl)-2-hydroxy-*N,N*-dimethylethanethioamide (0.5 g, 2.1 mmol) in Eaton's reagent (5 mL) were combined and heated rapidly to 60°C and left for 1 h. After cooling to room temperature over 2 hours the mixture was dropwise addition into prechilled aqueous sodium hydroxide (2 N, 16.25 mL) with constant stirring. This solution was then extracted with methyl *tert*-butyl ether (5 x 20 mL) and the combined organic extracts were dried (MgSO$_4$) and the solvent removed *in vacuo* to give a yellow solid. This was purified by flash chromatography with a gradient of 0-2% diethyl ether in hexane and gave 0.2 g of yellow solid containing an impurity, this was triturated with hexane to leave a colourless solid (0.155 g, 34%) of the title compound; δ_H (300 MHz, CDCl$_3$) 6.70-6.52 (2H, m, ArH), 6.12-6.10 (1H, m, ArH) 3.90 (3H, s, OCH$_3$) and 3.2 (6H, s, N(CH$_3$)$_2$), M+H = 226.1.

c) 7-Fluoro-4-methoxy-1-benzothiophen-2(3*H*)-one
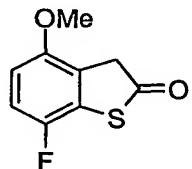
To a solution of *N*-(7-fluoro-4-methoxy-1-benzothien-2-yl)-*N,N*-dimethylamine (1.73 g, 7.7 mmol) in THF (25 mL) was added aqueous hydrochloric acid (1 N, 25 mL) and this was heated to 80°C for 3 h. After cooling to room temperature then extracted with ether (100 mL), dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give a yellow solid. The residue was purified by flash chromatography in 5% ethyl acetate in hexane to give (1.3 g, 84%) of the title compound; δ_H (300 MHz, CHCl$_3$) 6.97-6.87 (1H, m, ArH), 6.62-6.55 (1H, m, ArH), 3.91 (2H, s, CH$_2$) and 3.85 (3H, s, OCH$_3$).

d) 7-Fluoro-4-methoxy-1-benzothiophene

-39-

OMe
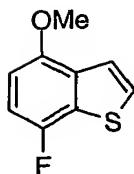
To a solution of 7-fluoro-4-methoxy-1-benzothiophen-2(3*H*)-one (1.06, 5.4 mmol) in dichloromethane (10 mL) at 0°C was added slowly a solution of diisobutylaluminium hydride in dichloromethane (1 M, 8.04 mL, 8.0 mmol). After 30 mins the reaction was quenched by careful addition of aqueous hydrochloric acid (6 N, 25 mL). The mixture was concentrated to remove the dichloromethane, the aqueous residue was then stirred at 35°C for 2 h. The aqueous solution was extracted with diethyl ether (3 x 50 mL), washed with aqueous sodium hydroxide (2 N, 50 mL), brine (50 mL) then dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give the title compound (0.86 g, 87%) as a purple oil; δ_H 7.44-7.38 (1H, m, ArH), 7.36-7.24 (1H, m, ArH), 6.88-6.80 (1H, m, ArH), 6.57-6.49 (1H, m, ArH) and 3.82 (3H, s, OCH$_3$).

7-Fluoro-4-methoxy-1-benzothiophene

a) 2,3-Difluoro-6-methoxybenzaldehyde

OMe
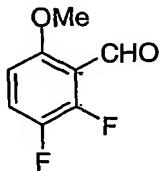
A solution of lithium diisopropylamide, 2M in THF/n-heptane (171 mL, 341 mmol) was further diluted with dry THF (250 mL) and cooled under nitrogen to -75°C. 3,4-Difluoroanisole (46.8 g, 325 mmol) in dry THF (100 mL) was added dropwise and the mixture stirred at -75°C for 1h. Dry *N,N*-dimethylformamide (27.6 mL, 358 mmol) was added dropwise and the mixture stirred for 10 mins at -70°C. Acetic acid (30 mL) and water (400 mL) were added, warming the temperature to 10°C. Extracted into diethyl ether (2 x 300 mL). Combined extracts were washed with water (250 mL), aqueous hydrochloric acid (0.2 N, 400 mL) and brine (2 x 250 mL), dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give a red/orange oil which crystallised. Purification was by recrystallisation from diethyl ether/petroleum ether 40-60 to give (53.0g, 95%) of solid;

-40-

δ_H (300 MHz, CDCl$_3$) 10.40 (1H, s, CHO), 7.37 (1H, q, ArH), 6.71 (1H, m, ArH), and 3.93 (3H, s OCH$_3$).

b) Methyl 7-fluoro-4-methoxy-1-benzothiophene-2-carboxylate
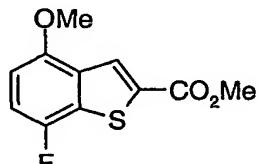
Methyl thioglycolate (28.8 mL, 320 mmol) was added under nitrogen to a solution of triethylamine (86.6 mL) in dry *N,N*-dimethylformamide (220 mL) at 80°C. Stirred at 100°C for 15 mins. A solution of 2,3-difluoro-6-methoxybenzaldehyde (55.1 g, 320 mmol) in *N,N*-dimethylformamide (80mL) was added and the mixture heated at 130°C for 3h. Allowed to cool then poured onto ice-water (2L). The resulting yellow solid was filtered, washing with water (2 x 200 mL). Dried under vacuum over phosphorus pentoxide at room temperature overnight to give the title compound (67.2g, 87%); δ_H (300 MHz, CDCl$_3$) 8.20 (1H, d, ArH), 7.06 (1H, t, ArH), 6.68 (1H, m, ArH) and 3.99 (6H, s, OCH$_3$ and CO$_2$CH$_3$).


c) 7-Fluoro-4-methoxy-1-benzothiophene-2-carboxylic acid
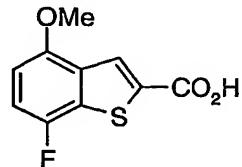
A mixture of methyl 7-fluoro-4-methoxy-1-benzothiophene-2-carboxylate (67.2 g, 280 mmol), sodium hydroxide (45 g, 1.12 mol), methanol (800 mL) and water (400 mL) were stirred at ambient overnight. The methanol was evaporated and the mixture cooled to 0°C. Acidified with concentrated hydrochloric acid and stirred for 20 mins. The yellow solid was filtered, washing with water (3 x 100 mL). Dried under vacuum at 45°C overnight, over phosphorus pentoxide to give the title compound (61.4 g, 97%); δ_H (300 MHz, d$_6$-DMSO) 8.10 (1H, d, ArH) 7.44 (1H, t, ArH) 7.00 (1H, m, ArH) and 4.02 (3H, s, OCH$_3$).


d) 7-Fluoro-4-methoxy-1-benzothiophene
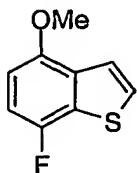
A mixture of 7-fluoro-4-methoxy-1-benzothiophene-2-carboxylic acid (61.4 g, 271

mmol) and copper powder (22.4 g, 352 mmol) in quinoline (500 mL) was heated at 190°C

for 1h. Cooled to ambient and poured onto aqueous hydrochloric acid (2 N, 750 mL).

Stirred with ethyl acetate (500 mL) for 15 minutes. Filtered through Celite, washing with

ethyl acetate. The aqueous layer was extracted into ethyl acetate and the combined organic

layers were washed with aqueous hydrochloric acid (2 N, 500 mL), water (500 mL), brine

(500 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Purified by column

chromatography, eluting silica gel with *iso*-hexane/diethyl ether 0-5% to give the product

as a brown oil which crystallised to give the title compound (41.3g, 84%); δ_H (300 MHz,

$CDCl_3$) 7.51 (1H, m, ArH), 7.38 (1H, d, ArH), 6.98 (1H, t, ArH), 6.65 (1H, dd, ArH) and

3.92 (3H, s, OCH_3).

3-Chloro-4-fluoro-7-methoxy-1-benzothiophene

a) (2E)-3-(2-Fluoro-5-methoxyphenyl)-2-propenoic acid
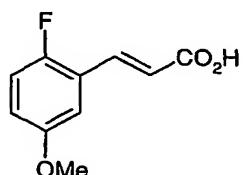
A solution of 2-fluoro-5-methoxybenzaldehyde (10.00 g, 64.9 mmol), malonic acid (13.4

g, 128.8 mmol), piperidine (2.00 mL) and pyridine (100 mL) was heated to 110°C for 2h.

After this time the solvent was removed *in vacuo* and the residue taken up in ethyl acetate

and washed with aqueous hydrochloric acid (2N, 100 mL). The organic solvent was dried

($MgSO_4$) and the solvent evaporated *in vacuo*. The solid was recrystallised from hot

etahanol to give a white solid (12.2 g, 95%); δ_H (300 MHz, DMSO) 7.60 (1H, d, J 7Hz,

CH=C), 7.31-7.28 (1H, m, Ar), 7.20 (1H, t, Ar), 7.07-6.97 (1H, m, Ar), 6.62 (1H, d, J

8Hz, CH=CH), 3.80 (3H, s, OCH_3).

-42-

b) Methyl 3-chloro-4-fluoro-7-methoxy-1-benzothiophene-2-carboxylate
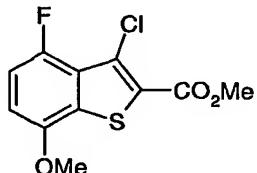
Thionyl chloride (3.7 mL, 50.8 mmL) was added to a stirred solution of (2E)-3-(2-fluoro-5-methoxyphenyl)-2-propenoic acid (2.5 g, 12.7 mmol) and pyridine (100 µl). The resulting yellow suspension was stirred at 120°C for 2 h before being allowed to cool to room temperature. The mixture was diluted with dichloromethane (50 mL) and concentrated *in vacuo*. The resulting yellow solid was taken up in methanol (100 mL) and heated to 70°C for 1hr. After this time the solvent was removed *in vacuo* to leave a white solid (984 mg, 28%); δ_H (300 MHz, CDCl$_3$) 7.09-6.98 (1H, m, Ar), 6.80-6.71 (1H, dd, Ar), 3.98 (6H, s, OCH$_3$ and CO$_2$CH$_3$).

c) 3-Chloro-4-fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid.
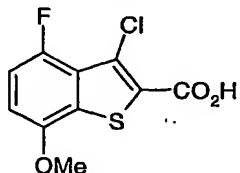
Methyl 3-chloro-4-fluoro-7-methoxy-1-benzothiophene-2-carboxylate (2.00 g, 7.29 mmol) was suspended in THF:H$_2$O [10:1] and lithium hydroxide (260 mg) added, the resulting suspension was heated to 40°C for 1 h. After cooling to room temperature the mixture was extracted with diethyl ether (50 mL) and the aqueous phase acidified to pH 2 and the solid collected by filtration and vacuum dried to give a white solid (1.09 g, 58%); δ_H (300 MHz, DMSO) 7.32-7.20 (1H, m, Ar), 7.15-7.05 (1H, dd, Ar), 3.98 (3H, s, OCH$_3$).

d) 3-Chloro-4-fluoro-7-methoxy-1-benzothiophene.
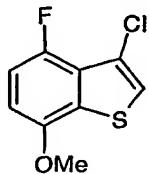
-43-

A mixture of 3-chloro-4-fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid (1.09 g, 4.19 mmol) and diazobicycloundecane (DBU) (2 mL) in dimethylacetamide (15 mL) was heated in a sealed vessel in a microwave (300W, 100%) for 1 h. After cooling to room temperature the mixture was diluted with diethyl ether (100 mL) and washed with brine (2 x 100 mL). The organic phase was dried (MgSO$_4$) and the solvent removed *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:diethyl ether [10:1] to yield a white solid (520mg, 57%); δ_H (300 MHz, CDCl$_3$) 7.24 (1H, s, Ar), 7.05-6.92 (1H, m, Ar), 6.70-6.60 (1H, dd, Ar), 3.96 (3H, s, OCH_3).

## 4-Fluoro-7-methoxy-3-methyl-1-benzothiophene

1-[(5-fluoro-2-methoxyphenyl)thio]acetone (1.00 g, 4.67 mmol) was added to a stirred solution of polyphosphoric acid (2.00 g) and chlorobenzene (70 mL). The resulting solution was stirred rapidly at 160°C for 18 h. After this time the solution was allowed to cool to room temperature and washed with water (50 mL). The aqueous phase was extracted with dichloromethane (3 x 30 mL) and the combined organic extracts dried (MgSO$_4$). The solvent was removed *in vacuo* and the residue purified by flash chromatography eluting silica gel with hexane:ethyl acetate [10:1] to give a pale yellow oil (700 mg, 76%); R$_f$ = 0.72 in hexane:ether [10:1]; δ_H (300 MHz, CDCl$_3$) 7.44-7.20 (1H, m, Ar), 6.99-6.82 (1H, m, Ar), 6.62-6.55 (1H, dd, Ar), 3.95 (3H, s, OCH_3), 2.56 (3H, s, CH_3).

Similarly prepared was

7-Fluoro-4-methoxy-3-methyl-benzothiophene as an oil (1.43 g, 91%); δ_H (300 MHz, CDCl$_3$) 7.22 (1H, s, ArH), 6.89-6.81 (1H, m, ArH), 6.59-6.52 (1H, m, ArH), 3.81 (3H, s, OCH$_3$) and 2.56 (3H, s, CH$_3$).

## 2-Fluoro-7-methoxy-1-benzothiophene

-44-
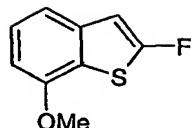
A solution of 1-benzothien-7-yl methyl ether (230 mg, 1.40 mmol) in dry THF (5 mL) was added dropwise to a freshly prepared solution of 2,2,6,6 tetramethyl-lithio-piperidine (1.68 mmol) in THF (10 mL) at -78°C. The resulting solution was stirred at this temperature for 30 mins before perchloryl fluoride gas was condensed into the reaction. After the strong exotherm had stopped the mixture was allowed to stir at -78°C for a further 30mins. After this time the reaction was quenched with NH_4Cl (sat, 20 mL) and diluted with diethyl ether. The organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane to yield a colourless oil (128 mg, 50%); δ_H (300 MHz, $CDCl_3$) 7.32-7.20 (2H, m, Ar), 6.80-6.62 (2H, m, Ar), 3.95 (3H, s, OCH_3).

Similarly prepared was

2-Fluoro-4-methoxy-1-benzothiophene as a colourless oil (420 mg, 48%); δ_H (300 MHz, $CDCl_3$) 7.32-7.20 (2H, m, Ar), 6.80-6.62 (2H, m, Ar), 3.95 (3H, s, OCH_3).

7-Methoxy-1-benzothiophene-2-carbonitrile
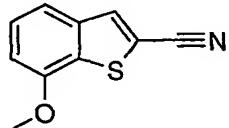
To a solution of 1-benzothien-7-yl methyl ether (1 g, 6.1 mmol) in THF (12 mL) at -78°C was added a solution of 2.5 M n-butyllithium in hexanes (2.9 mL, 7.3 mmol). After stirring for 1.5 hr this solution was added dropwise to a solution of tosyl cyanide (1.66 g, 9.1mmol) in THF (8 mL), this was left stirring at -78°C for 0.5 hr and then warmed to room temperature. After 16 h this was poured onto ice-water and extracted with dichloromethane (3 x 50 mL). The combined organic extracts were washed with water, dried ($MgSO_4$) and the solvent removed *in vacuo* to give an oil. This was purified by flash chromatography with a gradient of 0-30% ethyl acetate in hexane to give the title

compound (0.31 g, 27%); δ_H (300 MHz, CDCl$_3$) 7.79 (1H, s, 3-ArH), 7.46-7.38 (1H, m, 4-ArH), 7.37-7.31 (1H, m, 5-ArH), 6.88-6.82 (1H, m, 6-ArH) and 3.94 (3H, s, OCH$_3$). Starting material 1-benzothien-7-yl methyl ether was recovered from the reaction (0.56 g, 56%); δ_H (300 MHz, CDCl$_3$) 7.45-7.29 (4H, m, ArH), 6.75 (1H, m, ArH), 4.00 (3H, s, OCH$_3$).

7-Methoxy-1-benzothiophene-2-carbonitrile

a) 2-Iodo-7-methoxy-1-benzothiophene
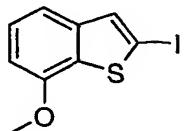
In a 4 L mechanically stirred reactor, a solution of 1-benzothien-7-yl methyl ether (105 g , 0.64 mol) in anhydrous THF (2 L) is cooled down to –74°C. *n*-Butyl lithium in hexane is added (2.5 N, 285 mL, 0.71 mol) within 45 min, keeping temperature below –70°C. The mixture is stirred 30 min at -78°C and a solution of iodine (179 g, 0.70 mol) in anhydrous THF (1L) is added within 1 h, keeping temperature below -70°C. After addition, the mixture is allowed to come up to room temperature over 2 h and brine (500 mL) is added. The layers are roughly separated and the organic layer is partially evaporated. Additional brine (200 mL) is added to the residual aqueous layer (mixed with some THF). After decantation, the organic and aqueous layers are separated. The aqueous layer is extracted with ethyl acetate (500 mL). The organic layers are pooled, washed with aqueous sodium thiosulphate, dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give the crude iodo derivative (173.6 g, 93%) The solid is recrystallized from isopropanol (150 mL) to give pure compound (145.5g, 88%); δ_H (600 MHz, CDCl$_3$) 7.51 (s, 1 H), 7.32 (d(br), *J*=7.89 Hz, 1 H), 7.26 (t, *J*=7.89 Hz, 1 H), 6.72 (d(br), *J*=7.89 Hz, 1 H), 3.97 (s, 3 H).

b) 7-Methoxy-1-benzothiophene-2-carbonitrile
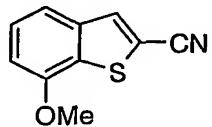
-46-

A solution of 2-iodo-7-methoxy-1-benzothiophene (10.0g, 0.35 mol), copper (1) cyanide (6.17 g, 0.68 mol) and anhydrous *N,N*-dimethylformamide (40 mL) are warmed to 130°C. After 2.5 h at 130°C no starting material is detectable as measured by HPLC at 220 nm. The reaction is cooled to 40°C and a solution 25% v/v ethylenediamine in water (30 mL)

5   and toluene (20 mL) are added. The mixture is stirred to room temperature. Additional toluene (30 mL) is added and the heterogeneous mixture is filtered. The layers of the mother liquors are separated and the aqueous layer is extracted with toluene (3 x 50 mL). The combined organic extracts were washed with water (2 x 50 mL), dried (MgSO$_4$) and the solvent evaporated *in vacuo* to give the title compound (5.78 g, 88%), which was used

10   without further purification; δ_H (300 MHz, CDCl$_3$) 7.79 (1H, s, 3-ArH), 7.46-7.38 (1H, m, 4-ArH), 7.37-7.31 (1H, m, 5-ArH), 6.88-6.82 (1H, m, 6-ArH) and 3.94 (3H, s, OCH$_3$).


4-Methoxy-1-benzothiophene-2-carbonitrile
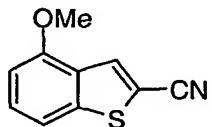
15   (Ref: Cheutin et al.; C.R.Hebd.Seances Acad.Sci; 261; 1965; 705.) A solution of 4-methoxy-1-benzothiophene-2-carboxylic acid (1.19g, 5.7 mmol) in pyidine (25 mL) at 0°C was treated with methanesulfonyl chloride (0.49 mL, 6.3 mmol) keeping the temperature at 0°C, stirring was continued for 2 h. Ammonia gas was then bubbled through the mixture for 5 minutes, followed by nitrogen for 10 mins. The reaction

20   mixture was then treated with a large excess of mesyl chloride (4.43 mL, 57 mmol) and stirred for 16 h at room temperature. The solvent evaporated *in vacuo* to give a brown residue which was purified by flash chromatography with 10% ethyl acetate in hexane to give a colourless solid (800 mg, 74%); δ_H (300 MHz, CDCl$_3$) 8.00 (1H, s, 3-ArH), 7.48-7.35 (2H, m, ArH), 6.80-6.77 (1H, m, ArH) and 3.92 (3H, s, OCH$_3$).

25

4-Fluoro-7-methoxy-1-benzothiophene-2-carbonitrile

a) 4-Fluoro-7-methoxy-1-benzothiophene-2-carboxamide

-47-

F

—CONH₂

S

OMe
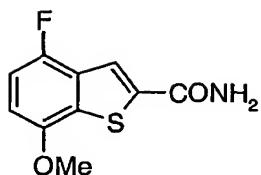
A solution of 4-fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid (1.5 g, 6.6 mmol) in thionyl chloride (4 mL) was heated to 50°C for 30 minutes, then the solvent removed *in vacuo*. The residue was taken up in dichloromethane (60 mL) and methanol (0.5 mL) and then added to a solution of concentrated ammonium hydroxide (40 mL) and dichloromethane (40 mL) at 5°C. After 10 min the solution was warmed to room temperature and stirred for 2 h. The dichloromethane was evaporated *in vacuo* and the solid filtered to give a pale browm solid (0.75 g, 51%); δ_H (300 MHz, D₄- Methanol) 7.95 (1H, s, 3-ArH), 7.02-6.92 (1H, m, ArH), 6.83-6.77 (1H, m, ArH), 3.89 (3H, s, OCH₃) and 3.23-3.28 (2H, m, CONH₂).

b) 4-Fluoro-7-methoxy-1-benzothiophene-2-carbonitrile

F

—CN

S

OMe
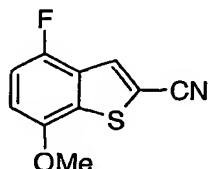
A solution of 4-fluoro-7-methoxy-1-benzothiophene-2-carboxamide (0.756 g, 3.3 mmol) in phosphorus oxychloride (6.2 mL, 6.6 mmol) was refluxed for 3 h then cooled and the solvent evaporated *in vacuo* to give the title compound. Purified by flash chromatography with a gradient of 0-30% ethyl acetate in *iso*-hexane to give a colourless solid (0.635 g, 91%); δ_H (300 MHz, CDCl₃) 7.95 (1H, s, 3-ArH), 7.10-7.01 (1H, m, ArH), 6.85-6.77 (1H, m, ArH) and 3.98 (3H, s, OCH₃).

4-Cyano-7-methoxy benzo[b]thiophene.

O

S

CN
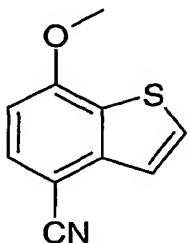
-48-

To a stirred solution of 4-bromo-7-methoxy benzo[b]thiophene (1.05 g, 4.32 mmol, 1 equiv.) in dry DMF (40 mL) was added copper(I)cyanide (3.885 g, 43.4 mmol, 10 equiv.) and the reaction mixture was heated at 150°C overnight. The reaction mixture was cooled to ~120 °C and then solid iron(III)chloride (1.58 g, 9.74 mmol) was added followed by 1

5   N HCl (CAUTION: HCN evolution – perform in a well vented hood!) The reaction mixture was heated at ~100°C for 2 hr before cooling to room temperature. Water, brine, and ethyl acetate were added and the layers were separated. The organic layer was washed with brine (3 times), dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude residue thus obtained was purified via

10 medium pressure liquid chromatography eluting with 10% ethyl acetate/90% hexanes to afford the title compound (564 mg, 69%) as a colorless solid; δ_H (400 MHz, CDCl$_3$) 4.06 (3H, s), 6.81 (1H, d, J = 8 Hz), 7.56 (1H, d, J = 6 Hz), 7.65 (1H, d, J = 6 Hz), 7.71 (1H, d, J = 8 Hz).

Ref: (J. Chem. Soc. Perkin Trans 1 **1983**, 2973).

15

6-Methoxy-1-benzothiophene-2-carbonitrile

a) O-(2-Formyl-5-methoxyphenyl) dimethylthiocarbamate
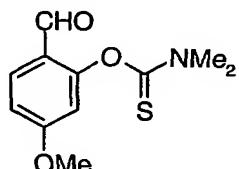
A solution of N, N-dimethylcarbamoyl chloride (4.46 g, 35.7 mmol) in THF (20 mL) was

20 added over 15 minutes to a stirred cooled (0°C), solution of 2-hydroxy-4-methoxybenzaldehyde (5 g, 32.9 mmol) and potassium hydroxide (2 g, 35.7 mmol) in water (25 mL) such that the temperature did not rise above 10°C. The mixture was stirred for 10 minutes at room temperature then extracted with ethyl acetate (3 x 50 mL), the combined organic layers were washed successively with 2M sodium hydroxide (100 mL),

25 2N hydrochloric acid (100 mL), brine (100 mL) then dried (MgSO$_4$) and the solvent removed *in vacuo* to give a yellow solid (6.8 g, 86%) which was used without further purification; δ_H (300 MHz, CDCl$_3$) 7.88-7.80 (1H, m, ArH), 7.95-7.85 (1H, m, ArH), 6.65-6.60 (1H, m, ArH), 3.88 (3H, s, OCH$_3$), 3.46 (3H, s, N(CH$_3$)$_2$) and 3.40 (3H, s, N(CH$_3$)$_2$).

-49-

b) *S*-(2-Formyl-5-methoxyphenyl) dimethylthiocarbamate

$$\text{CHO} \quad \text{S} \quad \text{NMe}_2 \quad \text{O} \quad \text{OMe}$$
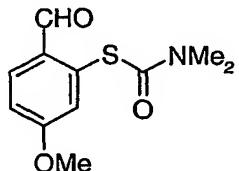
A pre-warmed solution of *O*-(2-formyl-5-methoxyphenyl) dimethylthiocarbamate (2 g,
8.3 mmol) in diphenyl ether (4 mL) was added to diphenyl ether (36 mL) at 230°C. The
mixture was heated at 230°C for 1.5 h. The reaction mixture was loaded neat onto flash
chromatography column and solvent eluted with *iso*-hexane. Product eluted with a
gradient of 0-30% ethyl acetate in *iso*-hexane. Further purified by flash chromatography
with a gradient of 0-20% ethyl acetate in dichloromethane, followed by triturating with
iso-hexane gave a solid which was recrystallised from ethyl acetate hexane to give the
title compound (1.21 g, 61%); δ_H (300 MHz, CDCl$_3$) 8.04-7.95 (1H, m, ArH), 7.09-6.99
(2H, m, ArH), 3.89 (3H, s, OCH$_3$), 3.16 (3H, br. s, N(CH$_3$)$_2$) and 3.02 (3H, br. s,
N(CH$_3$)$_2$).

c) 6-Methoxy-1-benzothiophene-2-carbonitrile

$$\text{MeO} \quad \text{S} \quad \text{CN}$$
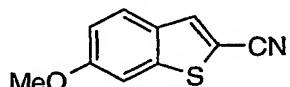
(Ref: Gallagher, T; Pardoe, D.A.; Porter, R.A.; Tetrahedron Lett.; 2000, 41(28), 5415 -
5418.) To a solution of *S*-(2-formyl-5-methoxyphenyl) dimethylthiocarbamate (1.08 g, 4.5
mmol) in water (4 mL) and methanol (8 mL) was added sodium hydroxide (199 mg, 4.9
mmol), this was heated to reflux for 16 hours. The reaction was cooled to room
temperature and chloroacetonitrile (0.28 mL, 4.5 mmol) was added in one portion and the
mixture the stirred at room temperature for 1 hour. The methanol was removed *in vacuo*,
and water (10 mL) added. The aqueous layer was extracted with diethyl ether (3 x 20
mL), dried (MgSO$_4$) and the solvent removed *in vacuo*. The residue was purified by flash
chromatography with a gradient of 0-30% ethyl acetate in *iso*-hexane to give the title
compound as a colourless solid (390 mg, 46%); δ_H (300 MHz, CDCl$_3$) 7.80-7.70 (1H, m,

ArH), 7.29 (2H, m, ArH), 7.11-7.01 (1H, m, ArH) and 3.85 (3H, s, OCH₃). M+23 = 212.0. v_{max}/cm⁻¹ [film] 2213.50 (m).

1-Benzothiophen-4-ol

5   a) 5-Bromo-6,7-dihydro-1-benothiophen-4(5H)-one
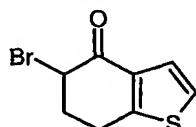
Ref: J. Chem. Res. (S) 1993, 192-193. Bromine (6.4 g, 40 mmol) in dry carbon tetrachloride containing a few drops of diethyl ether (20 mL) was added dropwise to a well stirred solution 6,7-dihydro-1-benothiophen-4(5H)-one (6.08 g; 40 mmol) in dry

10   diethyl ether (250 mL) allowing the solution to decolourise between additions and the temperature maintained at -10°C. Once the addition was complete, the solution was allowed to warm slowly to room temperature. Water (200 mL) was slowly added and the mixture transferred to a separating funnel with ether (100 mL). The organic phase was washed with water (100 mL), dried over magnesium sulfate and evaporated to an oil

15   which solidified on standing (8.56 g, 88%), this material was used without further purifictaion. δ_H (300 MHz, CD₃OD) 7.36 (1H, d, Ar) 7.30 (1H, d, Ar), 4.63 (1H, t, CH) 3.20 (2H, m, CH₂), 2.56 (2H, m, CH₂).

b) 1-Benzothiophen-4-ol
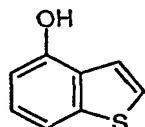
20

5-Bromo-6,7-dihydro-1-benothiophen-4(5H)-one (11.4 g, 50 mmol), lithium bromide (10g) and lithium carbonate (7.4 g) were refluxed for 3 h in dry N,N-dimethylformamide (250 mL) under nitrogen. The solvent was evaporated in vacuo and the residue treated with cold aqueous hydrochloric acid (1 N, 250 mL). Extracted into diethyl ether (3 x 200

25   mL). The ethereal layer was extracted with 10% aqueous sodium hydroxide solution (2x). Combined aqueous layers were acidified and extracted into ether. Dried over magnesium sulfate and evaporated to an oil. Purified by chromatography eluting silica gel with ethyl acetate – hexane (4-6%). Combined fractions were evaporated to a light yellow oil which

-51-

crystallised on standing, this material was triturated with cyclohexane to give white plates (3.6 g; 51%). δ_H (300 MHz, CD$_3$OD) 7.45 (1H, d, Ar), 7.30-7.40 (2H, m, Ar), 7.12 (1H, m, Ar), 6.70 (1H, m, Ar).

5-Fluoro-benzo[b]thiophen-4-ol

a)   5-Fluoro-6,7-dihydro-5H-benzo[b]thiophen-4-one
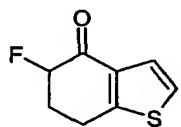
Add trimethylsilyl trifluoromethanesulfonate (18.5 mL, 102 mmol, 1.3 equiv.) over 5 minutes to a cold (0°C) stirred solution of solution 6,7-dihydro-1-benothiophen-4(5H)-one (12.04 g, 79.1 mmol, 1 equiv.) and triethylamine (33 mL, 237 mmol, 3 equiv.) in dry dichloromethane (200 mL). Stir the reaction mixture at 0°C for 5 minutes and then warm to room temperature for 1h10min before adding water. Separate the layers, extract the aqueous layer twice with dichloromethane, dry the combined organic extracts over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Dissolve the resulting crude silylenol ether in anhydrous dimethylformamide (200 mL), place the reaction mixture in a room temperature water bath, and add 1-(chloromethyl)-4fluoro-1,4-diazoniabicyclo[2.2.2]octanebis(tetrafluoroborate) (Selectfluor^{TM}) (31.06 g, 87.7 mmol, 1.1 equiv.) in one portion. Stir the reaction mixture at room temperature for 15 minutes then add tetrabutylammonium fluoride (1M in THF, 87 mL, 87 mmol, 1.1 equiv.) and stir at room temperature for 1 hr before adding water and ethyl acetate. Separate the layers, extract the aqueous layer three times with ethyl acetate, wash the combined organic extracts successively with water (2 times) then 0.1N HCl (2 times), dry the combined organic extracts over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resultant crude material by medium pressure liquid chromatography eluting with 0 to 20% ethyl acetate in hexanes to afford the title compound as an orange solid (9.725 g, 72%). δ_H (400 MHz, CDCl$_3$) 2.41-2.54 (m, 1H), 2.56-2.67 (m, 1H), 3.10-

-52-

3.20 (m, 1H), 3.23-3.33 (m, 1H), 5.12 (ddd, 1H, J = 47, 11, 4 Hz), 7.15 (d, 1H, J = 6 Hz),
7.41 (d, 1H, J = 6 Hz).

b)   5-Bromo-5-fluoro-6,7-dihydro-5H-benzo[b]thiophen-4-one
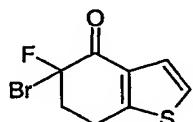
Add a solution of bromine (10.08 g, 63.1 mmol, 1.1 equiv.) in dry carbon tetrachloride
(60 mL) dropwise via addition funnel over 1h20min to a cold (0°C) solution of 5-fluoro-
6,7-dihydro-5H-benzo[b]thiophen-4-one (9.72 g, 57.1 mmol, 1 equiv.) in dry diethyl ether
(300 mL). Once all the bromine is added warm to room temperature for 2 hours and add
more bromine (0.7 M in CCl₄, 7 mL, 4.9 mmol, 0.09 equiv.) and stir the reaction mixture
at room temperature for 1 hr before adding saturated aqueous sodium thiosulfate. The
layers are separated and the aqueous layer is extracted with diethyl ether (three times).
The combined organic extracts are dried over anhydrous magnesium sulfate, filtered, and
concentrated under reduced pressure. Purify the resultant crude material by medium
pressure liquid chromatography eluting with 0 to 20% ethyl acetate in hexanes to afford
the title compound (6.976 g, 49%). δ_H (400 MHz, CDCl₃) 2.67-2.78 (m, 1H), 2.93 (dddd,
1H, J = 14, 4, 4, 4 Hz), 3.24-3.31 (m, 2H), 7.21 (d, 1H, J = 6 Hz), 7.45 (d, 1H, J = 6 Hz).

c)   5-fluoro-benzo[b]thiophen-4-ol
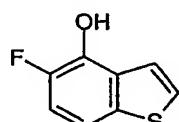
Heat a mixture of 5-bromo-5-fluoro-6,7-dihydro-5H-benzo[b]thiophen-4-one (6.97 g,
28.0 mmol, 1 equiv.), lithium carbonate (4.34 g, 58.7 mmol, 2.1 equiv.), and lithium
bromide (5.61 g, 64.6 mmol, 2.3 equiv.) in dry dimethylformamide (150 mL) at 150°C
(internal temperature) for 2 hours. Cool the reaction mixture to room temperature, add ice

water, and then carefully add 5N HCl until gas evolution ceases and the mixture is acidic. Add ethyl acetate, separate the layers, and extract the aqueous layer with ethyl acetate (3 times). Extract the combined organic extracts with 2N sodium hydroxide (2 times), reacidify the aqueous layers with hydrochloric acid, and then re-extract the aqueous layer

5   with dichloromethane (3 times). Dry the dichloromethane extracts over anhydrous magnesium sulfate, filter, and concentrate under reduced pressure. Purify the resultant crude material by medium pressure liquid chromatography eluting with 0 to 10% ethyl acetate in hexanes to afford the title compound as a colorless solid (3.738 g, 79%). δ_H (400 MHz, CDCl$_3$) 5.35 (br s, 1H), 7.13 (dd, 1H, J = 10, 8 Hz), 7.34 (ddd, 1H, J = 8, 4, 1

10   Hz), 7.42 (d, 1H, J = 6 Hz), 7.49 (dd, 1H, J = 6, 1 Hz).

Boron Tribromide Demethylation: General procedure

1-Benzothiophen-7-ol
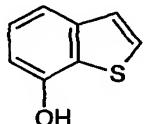
15

A solution of boron tribromide (115 µl, 1.21 mmol) was added dropwise at room temperature to a stirred solution of 1-benzothiophen-7-ol (200 mg, 1.21 mmol) in dry dichloromethane (10 mL). The resulting solution was allowed to stir at room temperature for a further 1 hr, after which the solvent was removed *in vacuo* and the residue taken up

20   in ethyl acetate (20 mL) and extracted with aqueous hydrochloric acid (2 N, 10 mL). The organic phase was dried (MgSO$_4$) and the solvent was removed *in vacuo*. The resulting dark yellow oil was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [4:1] to yield a white solid (68 mg, 38%); δ_H (300 MHz, CDCl$_3$) 7.50-7.39 (2H, m, Ar), 7.29-7.21 (1H, m, Ar), 7.21-7.15 (1H, m, Ar), 6.70 (1H, d, Ar), 5.15 (1H, bs,

25   OH).

Similarly prepared were

-54-

5-Fluoro-1-benzothiophen-7-ol as a brown crystalline solid (393 mg, 42%); δ_H (300 MHz, CDCl$_3$) 7.50 (1H, d, Ar), 7.28 (1H, d, Ar), 7.10 (1H, dd, Ar), 6.58 (1H, dd, Ar), 5.40 (1H, s, OH).

5   **4-Trifluoromethyl-1-benzothiophen-6-ol** as a brown crystalline solid (1.576 g, 62%); δ_H (300 MHz, CDCl$_3$) 7.50-7.40 (3H, m, Ar), 7.22 (1H, d, Ar), 5.30 (1H, bs, OH).

5-Fluoro-1-benzothiophen-4-ol as a white crystalline solid (0.074 g, 21%); δ_H (300 MHz, CDCl$_3$) 7.50 (1H, d, Ar), 7.42 (1H, d, Ar), 7.35 (1H, m, Ar), 7.10 (1H, t, Ar), 5.40

10   (1H, bs, OH).

4-Methyl-1-benzothiophen-7-ol as an oil (290 mg, 79%); δ_H (300 MHz, CDCl$_3$) 7.44 (1H, d, Ar), 7.32 (1H, d, Ar), 7.00 (1H, d, Ar), 6.61 (1H, d, Ar), 4.92 (1H, s, O*H*), 2.52 (3H, s, C*H*$_3$).

15

7-Fluoro-1-benzothiophen-4-ol as a solid (0.68 g, 79%); δ_H 7.45 (1H, m, ArH), 7.40 (1H, d, ArH), 6.90 (1H, t, ArH) and 6.74 (1H, m, ArH) and 5.00 (1H, br. s, OH).

3-Chloro-4-fluoro-1-benzothiophen-7-ol as a white solid (145 mg, 97%); δ_H (300 MHz,

20   CDCl$_3$) 7.25 (1H, s, Ar), 7.05-6.85 (1H, m, Ar), 6.72-6.61 (1H, dd, Ar).

3-Methyl-4-fluoro-1-benzothiophen-7-ol as a white solid (447 mg, 69%); δ_H (300 MHz, CDCl$_3$) 7.28 (1H, s, Ar), 6.99 (1H, s, O*H*), 6.90-6.78 (1H, m, Ar), 6.60 (1H, dd, Ar), 2.57 (3H, s, C*H*$_3$).

25

7-Fluoro-3-methyl-1-benzothiophen-4-ol as a solid (0.77 g, 70%); δ_H (300 MHz, CDCl$_3$) 7.26 (1H, s, ArH), 6.85-6.77 (1H, m, ArH), 6.58-6.50 (1H, m, ArH), 4.98 (1H, s, OH) and 2.65 (3H, s, CH$_3$).

30   **2-Fluoro-1-benzothiophen-7-ol** as a colourless oil (502 mg, 50%); δ_H (300 MHz, CDCl$_3$) 7.35-7.12 (3H, m, Ar), 6.72-6.63 (1H, dd, Ar).

2-Fluoro-1-benzothiophen-4-ol as a colourless oil (213 mg, 55%); δ_H (300 MHz, CDCl$_3$) 7.44 (1H, d, Ar), 7.41-7.12 (2H, m, Ar), 6.72-6.63 (1H, dd, Ar).

5   **7-Hydroxy-1-benzothiophene-2-carbonitrile** as a solid (3.9 g, 74%). δ_H (250 MHz, DMSO-D6) 6.98 (dd, J=7.87, 0.94 Hz, 1 H) 7.37 (t, J=7.87 Hz, 1 H) 7.49 (dd, J=7.87, 0.94 Hz, 1 H) 8.34 (s, 1 H) 10.87 (s, 1 H). Negative FIA: M-1 = 174.1.

4-Hydroxy-1-benzothiophene-2-carbonitrile as a solid (0.65 g, 95%) δ_H (300 MHz,

10   CDCl$_3$) 8.05 (1H, s, 3-ArH), 7.43-7.34 (2H, m, ArH), 7.80-7.75 (1H, m, ArH) and 5.68 (1H, br. s, OH). Negative FIA: M-1 = 174.1.

4-Fluoro-7-hydroxy-1-benzothiophene-2-carbonitrile as a solid (160 mg, 27%); δ_H (300 MHz, CDCl$_3$) 7.94 (1H, s, 3-ArH), 7.04-6.92 (1H, m, ArH), 6.85-6.76 (1H, m, ArH)

15   and 5.52 (1H, br. s, OH).

6-Hydroxy-1-benzothiophene-2-carbonitrile as a solid ; (0.36 g, 64%); δ_H (300 MHz, D$_4$-Methanol) 7.85 (1H, s, ArH), 7.73-7.62 (1H, m, ArH), 7.16 (1H, s, ArH) and 6.95-6.83 (1H, m, ArH). M+1 = 176.1.

20

1-Benzofuran-7-ol, synthesised from 7-methoxy-1-benzofuran, (Ref: Musser, J.H.; Chakraborty, U; Bailey, K; Sciortino, S; Whyzmuzis, C; Amin, D; Sutherland, C.A. J. Med. Chem. (1987), 30(1), 62-7.) as a solid (0.73 g, 82%); δ_H (300 MHz, CDCl$_3$) 7.61 (1H, d, Ar), 7.16 (2H, m, Ar), 6.82 (2H, m, Ar), 5.37 (1H, bs, -OH).

25

6-Fluoro-1-benzothiophene-7-ol
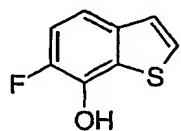
(Ref: Briner, K; Burkholder, T.P; Conway, R.G; Cunningham, B.E; Finley, D.R; Heinz, L.J; Jesudason, C.D; Kohlman, D.T; Liang, S.X; Xu, Y.C.   Preparation and use of serotonergic benzothiophenes. WO 0109126 A1. Chem. Abs. 134:162912).

To a solution of 7-bromo-6-fluoro-1-benzothiophene (0.2 g, 0.9 mmol) and
5   trimethylborate (0.2 mL, 1.8 mmol) at -78°C was added *tert*-butyllithium dropwise. After 10 mins at -78°C the reaction was quenched by pouring onto saturated ammonium chloride. This was extracted with ethyl acetate (3 x 10 mL) and the solvent removed *in vacuo* from the combined organic extracts. The residue was taken up in ethyl acetate (4 mL) and 10% aqueous hydrochloric acid (4 mL) was added, the mixture was stirred at
10   room temperature for 1 h. The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 x 10 mL). The combined organic extracts were dried (MgSO$_4$) and the solvent removed *in vacuo*. The residue was taken up in THF (10 mL), water (2 mL) and cooled to 0°C then 10% aqueous sodium hydroxide (2 mL) and 28% hydrogen peroxide (1 mL) were added to this and stirred for 0.5 h at 0°C. The mixture
15   was warmed to room temperature and stirred for 2 hours, before a acetic acid (3 mL) was added. The mixture was extracted with ethyl acetate (3 x 10 mL), dried (MgSO$_4$) and the solvent removed *in vacuo*, to give a purple solid. This was purified by flash chromatography with a gradient of 0-20% ethyl acetate in *iso*-hexane to give the title compound (56 mg, 38%); δ_H (300 MHz, CDCl$_3$) 7.41 (4H, m, ArH).

20

4-Cyano-7-hydroxy benzo[b]thiophene
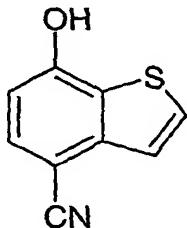
To a solution of 4-cyano-7-methoxy benzo[b]thiophene (450 mg, 2.38 mmol, 1 equiv.) in dry DMF (20 mL) was added sodium ethanethiolate (80% technical grade, 1.34 g, ~13
25   mmol, ~5 equiv.) and the reaction mixture was heated at 150°C for 2 hr. The mixture was cooled to room temperature and ethyl acetate and 1N HCl were added and the layers were separated. The aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with brine (3 times), dried over anhydrous magnesium sulfate,

filtered, and concentrated under reduced pressure. The acquired material thus obtained was purified via medium pressure liquid chromatography eluting with 30% ethyl acetate/70% hexanes to afford the title compound (414 mg, 99%) as a colorless solid; δ_H (400 MHz, CD$_3$OD) 6.80 (1H, d, J = 8 Hz), 7.47 (1H, d, J = 6 Hz), 7.64 (1H, d, J = 8 Hz),

5   7.83 (1H, d, J = 6 Hz).

1-Methyl-1H-indol-5-ol
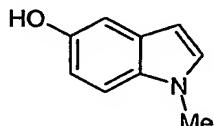
(Ref: Taborsky, R.G.; Delvigs, P; Palaic, D; Bumpus, F.M.   J. Med. Chem.   (1967),

10   10(3), 403-7).   To a solution of 5-benzyloxy-1-methyl-1H-indole (2 g, 8.9 mmol) in ethanol (20 mL) was added potassium hydroxide (0.62 g, 11.2 mmol).   The resulting solution was allowed to stir at room temperature for 10 mins before evaporating the ethanol *in vacuo*. The residue was taken up in acetone (75 mL) and sodium sulfate (6.4 g, 44.8 mmol) was added, followed by dimethyl sulfate (0.87 mL, 8.9 mmol) *via* syringe.

15   The solution was stirred for 0.5 h, then filtered and evaporated *in vacuo*. The resulting residue was then taken up in ethanol (50 mL) and 10% palladium on charcoal (0.4 g) was added.   This solution was stirred under a hydrogen atmosphere for 4 h, then filtered through celite and the solvent evaporated *in vacuo*. This material was purified by flash chromatography, eluting silica gel with hexane:ethyl acetate (100:0 to 50:50) to give the

20   product. (0.48 g, 37%); δ_H (300 MHz, CDCl$_3$) 7.15 (1H, d, Ar), 7.01 (2H, m, Ar), 6.8 (1H, m, Ar), 6.32 (1H, m, Ar), 3.73 (3H, s, NCH$_3$).


1-Methyl-1H-indol-7-ol

a) 7-Benzyloxy-1-methyl-1H-indole

25
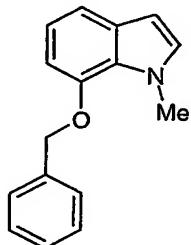
−58−

To a solution of 7-benzyloxy-1*H*-indole (Ref: Dobson, D; Todd, A; Gilmore, J. Synth. Commun. (1991), 21(5), 611-17) (1.29 g, 5.78 mmol) in ethanol (30 mL) was added potassium hydroxide (0.41 g, 7.23 mmol) and dichloromethane (5 mL) of to help solubilize the starting material. The resulting solution was allowed to stir at room

5   temperature for 10 min before evaporating the solvent *in vacuo*. The residue was taken up in acetone (75 mL) and sodium sulfate (4.9 g, 34.7 mmol) was added followed by dimethyl sulfate *via* syringe (0.62 mL, 6.3 mmol). The solution was stirred for 1 h, then filtered and evaporated *in vacuo*. This material was purified by flash chromatography, eluting silica gel with hexane: ethyl acetate (100:0 to 10:1) to give the product. (1.12 g,

10   82%); Mass spectrum (ion spray): m/z = 238.1 (M+1).


b) 1-Methyl-1*H*-indol-7-ol
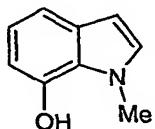
To a solution of 7-benzyloxy-1methyl-1*H*-indole in 20 mL of ethanol was added 10%

15   palladium on charcoal (0.2 g). This solution was stirred under balloon pressure hydrogen atmosphere for 4 h, then filtered through celite and concentrated *in vacuo*. This material was purified by flash chromatography, eluting silica gel with hexane:ethyl acetate (100:0 to 50:50) to give the product (0.58 g, 87%); Mass spectrum (TOF): m/z = 147.1 (M).

20   **Isoquinolin-4-ol**
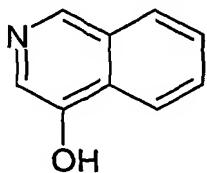
The title compound was prepared as described in *Tetrahedron*, **1963**, *19*, 827-832.


Isoquinolin-6-ol

25   a) 6-Methoxy-isoquinoline
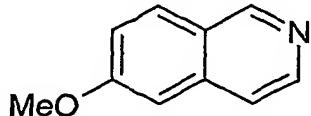
The title compound was prepared as described in *Synth. Commun.*, **1999**, *29*, 1617-1625.

b) Isoquinolin-6-ol
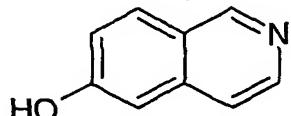
5   Heat a mixture of 6-methoxy-isoquinoline (2.1 g, 13.2 mmol) and pyridine hydrochloride (30 g) in a heavy walled screw cap sealed tube at 160^0C overnight. Cool to room temperature, add water and concentrated ammonium hydroxide to bring the pH of the mixture to 10-11, extract with ethyl acetate (4 times), wash the combined organic extracts with water (4 times), and concentrate under reduced pressure. Purification by medium

10   pressure liquid chromatography eluting with 0-3% of 2N NH_3/MeOH in dichloromethane afford the title compound (520 mg, 27%): δ_H (DMSO-d6, 400 MHz): 7.09 (s, 1H), 7.19 (dd, 1H, J = 9, 2 Hz), 7.56 (d, 1H, J = 6 Hz), 7.94 (d, 1H, J = 9 Hz), 8.29 (d, 1H, J = 6 Hz), 9.05 (s, 1H), 10.36 (s, 1H).

15   **[1,7]Naphthyridin-5-ol**

The title compound was prepared as described in *Liebigs Annalen Der Chemie*, **1979**, 443-445.

20   **5-Hydroxyisoquinoline**
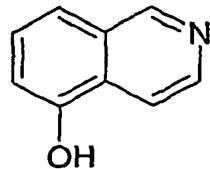
The title compound is commercially available and was purchased from the Aldrich Chemical Company.

-60-

5-Quinolinol

The title compound is commercially available and was purchased from the Aldrich Chemical Company.

Benzo[d]isothiazol-4-ol

a) 4-Methoxy-benzo[d]isothiazole
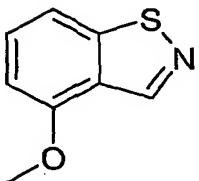
To a solution of 2-fluoro-6-methoxybenzaldehyde (2.0 g, 13.0 mmol) in 2-methoxyethanol (10 mL) in a sealed tube was added sulfur (416 mg, 13.0 mmol) and aqueous ammonium hydroxide (10 mL). The solution was heated to 160 degC for 18 h and was then cooled to rt. The reaction was partitioned between dichloromethane and water. The organic layer was separated and the aqueous layer was extracted with 2 x 50 mL dichloromethane. The combined organic phases were dried (MgSO$_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with EtOAc:hexane [0:100 to 4:6] to yield the title compound as an oil (1.51 g, 70%); Mass spectrum (ion spray): m/z = 165.9 (m+1).

b) Benzo[d]isothiazol-4-ol
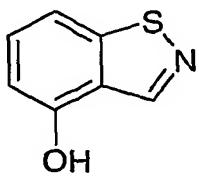
To a sealed tube was added 4-methoxy-benzo[d]isothiazole (760 mg, 4.60 mmol) and pyridine hydrochloride (5.5 g, 48 mmol). The reaction was heated to 150 °C for 18 h and was then cooled to rt. The mixture was partitioned between dichloromethane and water. The organic phase was separated and the aqueous layer was extracted with 2 x 30 mL

-61-

dichloromethane. The combined organic phases were dried (MgSO₄) and concentrated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with EtOAc:hexane [0:100 to 3:7] to yield the title compound as a solid (223 mg, 32%); Mass spectrum (ion spray): m/z = *151.9 (m+1)*.

5

7-methyl-benzo[d]isothiazol-4-ol

a) 7-Bromo-4-methoxy-benzo[d]isothiazole
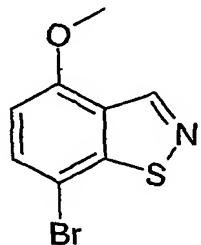
To a solution of 4-methoxy-benzo[*d*]isothiazole (prepared as described above) (1.0 g,

10   6.05 mmol) in carbon tetrachloride (20 mL) at 0°C was added bromine (310 μL, 6.05 mmol) in carbon tetrachloride (10 mL). The reaction was allowed to stir at 0°C for 3 h and was then warmed to rt. Saturated aqueous NaHCO₃ and dichloromethane were added and the organic phase was separated. The aqueous phase was extracted with 2 x 20 mL dichloromethane. The combined organic phases were dried (MgSO₄) and concentrated *in*

15   *vacuo*. The residue was purified by flash chromatography eluting silica gel with EtOAc:hexane [0:100 to 1:20] to yield the title compound (980 mg, 66%): δ$_H$ (300 MHz, CDCl₃): 9.09 (1H, s), 7.52 (1H, d, *J* = 8.1 Hz), 6.67 (1H, d, *J* = 8.4 Hz), 4.00 (3H, s).

b) 7-methyl-benzo[d]isothiazol-4-ol
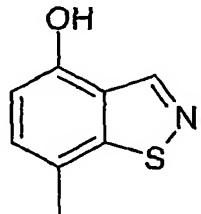
20

A solution of 7-bromo-4-methoxy-benzo[*d*]isothiazole (460 mg, 1.88 mmol), K₂CO₃ (780 mg, 5.64 mmol), and Pd(PPh₃)₄ (217 mg, 0.188 mmol) in 1,4-dioxane (5 mL) was added trimethylboroxine (290 μL, 2.07 mmol) and the solution was heated to 110°C for 18 h. The reaction was cooled to rt and diluted with water and dichloromethane. The

-62-

organic phase was separated and the aqueous phase was extracted with 2 x 30 mL

dichloromethane. The combined organic phases were dried (MgSO₄) and concentrated *in*

vacuo. The residue was purified by flash chromatography eluting silica gel with

EtOAc:hexane [0:100 to 1:10] to yield 4-methoxy-7-methyl-benzo[*d*]isothiazole (88 mg,

5   26%). A method similar to that described for the preparation of benzo[d]isothiazol-4-ol

(above) using 4-methoxy-7-methyl-benzo[*d*]isothiazole (88 mg, 0.491 mmol) and

pyridine hydrochloride (567 mg, 5 mol) gave the title compound (30 mg, 37%): δ$_H$ (300

MHz, CDCl₃): 8.99 (1H, s), 7.15 (1H, d, *J* = 7.5 Hz), 6.70 (1H, d, *J* = 7.5 Hz), 2.45 (3H,

s).

10

**Benzo[d]isothiazol-7-ol**

a)   2-Fluoro-3,N-dimethoxy-N-methyl-benzamide
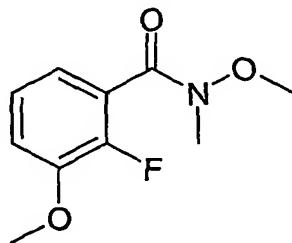
To a solution of 2-fluoro-3-methoxy-benzoic acid (5.0 g, 29.4 mmol) and PyBOP (13.7 g,

15   29.4 mmol) in 7:1 CH₂Cl₂:THF was added triethylamine (4.10 mL, 29.4 mmol) over a 10

min period. *N,O*-Dimethylhydroxylamine hydrochloride (2.87 g, 29.4 mL) was then

added and the reaction was allowed to stir at rt for 3 h. The reaction was then partitioned

between dichloromethane and water. The organic phase was separated and the aqueous

phase was extracted with 2 x 100 mL dichloromethane. The combined organic phases

20   were dried (MgSO₄) and concentrated *in vacuo*. The residue was taken up in ethyl

acetate and was washed successively with 1N HCl, saturated aqueous NaHCO₃, and brine.

The organic phase was again dried (MgSO₄) and concentrated *in vacuo* to yield the title

compound (2.30 g, 37%).

25   b)   2-Fluoro-3-methoxy-benzaldehyde

-63-
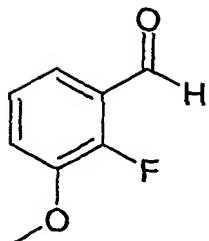
To a solution of 2-fluoro-3,*N*-dimethoxy-*N*-methyl-benzamide (2.30 g, 10.8 mmol) in
THF (20 mL) at –78°C was added 1M DIBAL-H in toluene (12 mL, 12 mmol). The
reaction stirred at –78°C for 3 h and then the remaining 1M DIBAL-H in toluene (4.2 mL,

5   4.2 mmol) was added to the reaction. The reaction was allowed to stir at –78°C for 30
min and was then warmed to rt. The reaction was quenched slowly with saturated
aqueous NH₄Cl. The organic phase was separated and the aqueous phase was extracted
with 2 x 50 mL ethyl acetate. The combined organic phases were washed successively
with 1N HCl and brine. The organic phase was dried (MgSO₄) and concentrated *in*

10   *vacuo*. The residue was purified by flash chromatography eluting silica gel with
EtOAc:hexane [0:100 to 1:1] to yield the title compound (1.41 g, 85%): δ_H (300 MHz,
CDCl₃): 10.38 (1H, s), 7.43-7.40 (1H, m), 7.24-7.15 (2H, m), 3.95 (3H, s).

    c)   7-methoxy-benzo[d]isothiazole
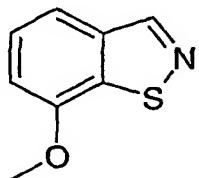
15

A method similar to 4-methoxy-benzo[d]isothiazole using 2-fluoro-3-methoxy-
benzaldehyde (410 mg, 2.66 mmol), sulfur (85 mg, 2.66 mmol), NH₄OH (5 mL), and 2-
methoxyethanol (5 mL) gave the title compound (60 mg, 14%); Mass spectrum (ion-
spray): m/z = 165.8 (m+1).

20

    d) Benzo[d]isothiazol-7-ol
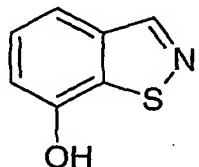
-64-

A method similar to that used in the preparation of benzo[d]isothiazol-4-ol using 7-methoxy-benzo[d]isothiazole (60 mg, 0.363 mmol) and pyridine hydrochloride (500 mg, 4.33 mmol) gave the title compound (26 mg, 47%); Mass spectrum (ion-spray): m/z = 151.9 (m+1).

5

4-Hydroxy-benzothiazole

a) 4-Methoxy-benzothiazole
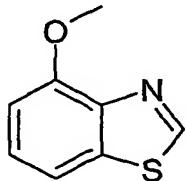
2-Amino-4-methoxy-benzothiazole (1.00 g, 5.54 mmol) was added to a stirred solution of
10   polyphosphoric acid (85%, 40 ml) at 60 °C. The resulting mixture was stirred at 60 °C until all the benzothiazole had dissolved. The resulting solution was then cooled to –10 °C and a solution of sodium nitrite (2.3 g, 33.3 mmol) in water (5 ml) was added so as to keep the internal temperature below –4 °C. After complete addition the resulting solution was added to a solution of hypophosphoric acid (50%, 15 ml) at 0 °C. After the evolution
15   of gas had ceased the mixture was diluted with water and basified with $NaHCO_3$ (sat). The aqueous solution was extracted with $CHCl_3$ (3 x 200 ml) with the combined organic extracts dried ($MgSO_4$) and the solvent removed *in vacuo*. The resulting solid was recrystallised from EtOH:H_2O to give an orange solid (300 mg).

20   The liquor was concentrated and purified by flash chromatography eluting silica gel with hexane:EtOAc [4:1] to hexane:EtOAc [1:1] to give a further 210mg of product. $R_f = 0.38$ in hexane:ether [1:1]; δ_H (300 MHz, $CDCl_3$) 8.91 (1H, s, CH), 7.53 (1H, d, Ar), 7.39 (1H, t, Ar), 6.93 (1H, d, Ar), 4.07 (3H, s, OCH_3).

25   b) 4-Hydroxy-benzothiazole

−65−

Boron tribromide (3.09ml, 1M solution in DCM, 3.09 mmol) was added dropwise at 0 °C to a stirred solution of 4-methoxy-benzothiazole (510 mg, 3.09 mmol) in dry DCM (30ml). The resulting solution was warmed to 40 °C and allowed to stir overnight. The resulting solution was concentrated *in vacuo* and diluted with water and HCl (2N). The

5   aqueous phase was neutralised to pH ~7 with NaHCO$_3$ and the solution extracted with EtOAc (3 x 100 ml) and the combined organic extracts dried (MgSO$_4$) and the solvent removed *in vacuo*. The resulting oil was purified by flash chromatography eluting silica gel with hexane:EtOAc [4:1] to hexane:EtOAc [7:3] to give the title compound as a tan solid (730mg, 80%); δ_H (300 MHz, CDCl$_3$) 7.59 (1H, s, C*H*), 7.46 (1H, dd, Ar), 7.36 (1H,

10   t, Ar), 7.02 (1H, dd, Ar).


Thieno[3,2-*c*]pyridin-7-ol
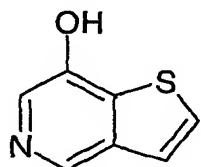
The title compound was prepared as described in Patent GB 2010249A.

15

Thieno[2,3-*c*]pyridin-4-ol
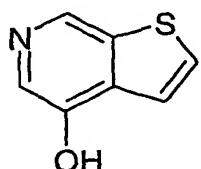
The title compound was prepared as described in Patent GB 2010249A.


20   ## 4-Fluoro-2,3-dihydrobenzo[b]thiophen-7-ol

a) 5-(2-Fluoro-5-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one

-66-

To a suspension of 2-fluoro-5-methoxybenzaldehyde (5.00 g, 32.46 mmol) and rhodanine (4.31 g, 32.46 mmol) in dry toluene (1000 mL) was added ammonium acetate (50 mg) and acetic acid (2 mL). The resulting suspension was allowed to stir at 120°C for 12 h under Dean-Stark apparatus before being allowed to cool and filtered. Resultant solid was washed with hexane and allowed to dry *in vacuo* to give an orange crystalline solid (8.00 g, 91%); δ_H (300 MHz, CDCl$_3$) 7.50 (1H, s, CH=C); 7.31 (1H, t, Ar), 7.20-7.11 (1H, m, Ar), 6.95-6.89 (1H, m, Ar), 3.80 (3H, s, OCH_3).

b) (2Z)-3-(2-Fluoro-5-methoxyphenyl)-2-mercapto-2-propenoic acid
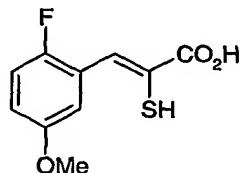
5-(2-Fluoro-5-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (8.00g, 9.7 mmol) was added in one portion to 25% w/v sodium hydroxide solution (40 mL). This was allowed stir at reflux for 1 h. After this time the reaction was allowed to cool to room temperature and poured onto water (50 mL). This was washed with dichloromethane (50 mL), and the aqueous layer acidified to pH 2 with aqueous hydrochloric acid (2 N, 50 mL) to give a white suspension. Product was extracted with ether (2 x 60 mL), dried (MgSO$_4$) and solvent removed *in vacuo* to give a white solid (6.71 g, 100%); δ_H (300 MHz, CD$_3$OD) 7.85 (1H, s, Ar), 7.46-7.35 (1H, m, Ar), 7.11 (1H, t, Ar), 7.01-6.75 (2H, m, CH=, and SH), 3.80 (3H, s, OCH_3).

c) 4-Fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid .
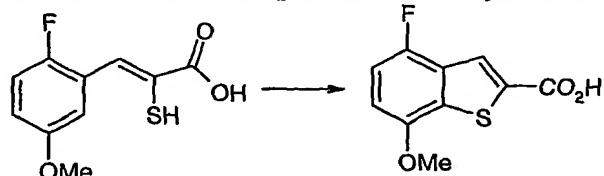
(2Z)-3-(2-Fluoro-5-methoxyphenyl)-2-mercapto-2-propenoic acid (1.00 g, 4.38 mmol) was added in one portion to a solution of iodine (1.66 g, 6.56 mmol) in dimethoxyethane (10 mL). This was heated in the microwave with 300W at 160°C for 10 mins. After this time the reaction was allowed to cool to room temperature and poured onto saturated

-67-

sodium metabisulphite (200 mL) and ether (400 mL). Ether layer was separated and product extracted with aqueous sodium hydroxide (2 N, 2 x 100 mL). This was then acidified to pH 2 with aqueous hydrochloric acid (2 N, 250 mL), and product extracted with ether (2 x 150 mL). The combined organic extracts were dried (MgSO₄) and

5   concentrated *in vacuo* to give a white solid (580 mg, 30%); δ_H (300 MHz, CD$_3$OD) 8.00 (1H, s, Ar), 7.30-7.19 (1H, m, Ar), 7.10-7.00 (1H, m, Ar), 3.95 (3H, s, OCH_3).

d) 4-Fluoro-7-methoxy-1-benzothiophene
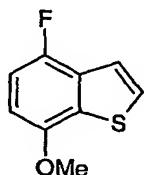
10   4-Fluoro-7-methoxy-1-benzothiophene-2-carboxylic acid (2.00 g, 8.84 mmol) was added in one portion to DBU (8 mL) and dimethyl acetamide (10 mL). This was heated in the microwave with 300W at 200°C for 1 h. The reaction mixture was allowed to cool and poured onto water (100 mL). Product was extracted with hexane (2 x 100 mL), washed with aqueous hydrochloric acid (2 N, 50 mL), aqueous sodium hydroxide (2 N, 50 mL),

15   and the combined organic extracts were dried (MgSO₄) and concentrated *in vacuo*. The residue was purified by flash chromatography eluting silica gel with hexane:ethyl acetate [96:4] to give an oil (1.12 g, 70%): δ_H (300 MHz, CDCl$_3$) 7.4 (2H, s, Ar), 6.9 (1H, t, Ar), 6.60 (1H, dd, Ar), 3.91 (3H, s, OCH_3).

20   e) 4-Fluoro-7-methoxy-2,3-dihydrobenzo[b]thiophene
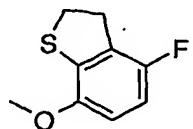
To a solution of 4-fluoro-7-methoxy-1-benzothiophene (1.55 g, 8.5 mmol, 1 eq) in trifluoroacetic acid (40 ml) was added triethylsilane (3.40 ml, 21.25 mmol, 2.5 eq). The mixture was heated to 60°C for 48 hours, then cooled to room temperature and the solvent

25   removed *in vacuo*. The crude product was purified by flash chromatography with a gradient of 40-60% chloroform in heptane to give 1.24 g, 80% recovered starting material

-68-

and 199 mg, 13% yield of the title compound as a colourless oil: δ_H (300 MHz, CDCl$_3$) 3.78-6.58 (2H, m, ArH), 3.82 (3H, s, CH$_3$) and 3.44-3.30 (4H, m, SCH$_2$CH$_2$).

f)   4-Fluoro-2,3-dihydrobenzo[b]thiophen-7-ol
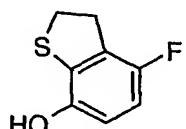
A BBr3 demethylation of 4-fluoro-7-methoxy-2,3-dihydrobenzo[b]thiophene similar to that described for 4-hydroxy benzothiazole affords the title compound as a brown solid 251 mg: δ_H (300 MHz, CDCl$_3$) 6.57-6.48 (2H, m, ArH), 4.67 (1H, br. s, OH) and 3.43-3.23 (4H, m, SCH$_2$CH$_2$).

3-(Benzyl-methyl-amino)-*N*-methoxy-*N*-methyl-propionamide
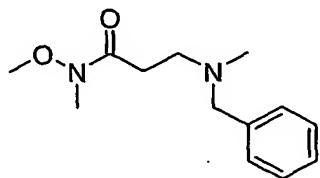
Add potassium carbonate (63.50 g, 459 mmol) to a stirred solution of *N*-benzylmethylamine (14.8 mL, 115 mmol), 3-bromo-*N*-methoxy-*N*-methyl-propionamide (22.47 g, 115 mmol, prepared according to Jacobi, P. A.; Blum, C. A.; DeSimone, R. W.; Udodong, U. E. S. *J. Am. Chem. Soc.* **1991**, *113*, 5384-5392), and anhydrous acetonitrile (460 mL). Heat the reaction to reflux under nitrogen for 3 hours. Cool the reaction to room temperature and filter the reaction through Celite®. Wash the Celite® with ethyl acetate and concentrate on a rotary evaporator to give the crude product. Purify the crude product by flash chromatography on silica gel eluting with 0.5% concentrated ammonium hydroxide/5% ethanol/chloroform to yield 22.31 g (82%) of 3-(benzyl-methyl-amino)-*N*-methoxy-*N*-methyl-propionamide: mass spectrum (ion spray) m/z = 237.1(M+1); ^{1}H NMR (CDCl$_3$) δ 7.31-7.21 (m, 5H), 3.65 (s, 3H), 3.53 (s, 2H), 3.17 (s, 3H), 2.80-2.76 (m, 2H), 2.67-2.63 (m, 2H), 2.23 (s, 3H).

1-(Benzyl-methyl-amino)-heptan-3-one
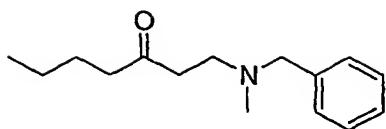
Add butyllithium (12.3 ml (1.6 M solution in hexane), 19.6 mmol) to a stirred solution of
3-(benzyl-methyl-amino)-N-methoxy-N-methyl-propionamide and anhydrous
tetrahydrofuran (70 ml) at –40 °C under N_2. Stir for 1 hr at –40 °C. Quench the reaction
5   with saturated ammonium chloride (25 ml), allow the reaction to reach room temperature
and add saturated sodium bicarbonate. Extract with ethyl acetate, wash with brine and dry
over sodium sulfate. Filter off and concentrate to give the crude product. Purify the
compound by flash chromatography, eluting with 2% ethanol/0.2% ammonium
hydroxide/chloroform to yield 2.41 g (81%) of 1-(benzyl-methyl-amino)-heptan-3-one:
10   mass spectrum (ion spray) m/z = 234 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.31-7.24 (m, 5H), 3.5
(s, 2H), 2.74-2.7 (m, 2H), 2.64-2.60 (m, 2H), 2.41 (t, 2H), 2.19 (s, 3H), 1.59-1.51 (m,
2H), 1.35- 1.28 (m, 2H), 0.91 (t, 3H).

1-(Benzyl-methyl-amino)-hexan-3-one

15
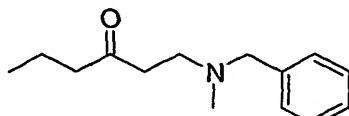
Using a method similar to that for 1-(Benzyl-methyl-amino)-heptan-3-one,
propylmagnesium chloride (2 M solution in diethyl ether) affords the title compound:
mass spectrum (ion spray) m/z = 220 (M+1): 1H NMR (CDCl$_3$) δ 7.35-7.24 (m, 5H),
3.51 (s, 2H), 2.74-2.69 (m, 2H), 2.66-2.61 (m, 2H), 2.39 (t, 2H), 2.20 (s, 3H), 1.67-1.57
20   (m, 2H), 0.92(t, 3H).

(4-Cyclohexyl-3-oxo-butyl)-methyl-carbamic acid *tert*-butyl ester
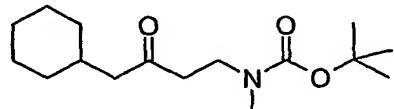
Add dropwise bromomethylcyclohexane (16.96 mL, 0.121 mol) to a stirred warm solution
25   of magnesium (granulae, 3.02 g, 0.124 mol), a crystal of iodine and tetrahydrofuran (130
mL). Heat the mixture to reflux for one hour. Add the Grignard reagent (30 mL) to a

stirred mixture of [2-(methoxy-methyl-carbamoyl)-ethyl]-methyl-carbamic acid *tert*-butyl ester (3.0 g, 12.0 mmol, prepared according to Blaney, P.; Grigg, R.; Rankovic, Z.; Thornton-Pett, M.; Xu, J. *Tetrahedron* **2002**, 1719-1737) and tetrahydrofuran (120 mL) kept at 0 °C under N_2. Stir the mixture for one h at 0 °C and for 2 h at room temperature.

5   Add more of the Grignard reagent (40 mL) and stir for another hour at room temperatur. Add saturated aqueous ammonium chloride and separated the layers. Extract the water layer with diethyl ether (x3). Dry the combined organic layers, filter and concentrate. Purify the crude residue by flash column chromatography, eluting with ethyl acetate/hexane (1:9) to yield 1.0 g of the title compound. ^{1}H NMR (CDCl$_3$) δ   3.46-3.40

10   (m, 2H), 2.85 (s, 3H), 2.68-2.60 (m, 2H), 2.29 (d, 2H), 1.87-1.60 (m, 6H), 1.46 (s, 9H), 1.33-1.07 (m, 3H), 0.99-0.86 (m, 2H).

1-(Benzyl-methyl-amino)-heptan-3-ol
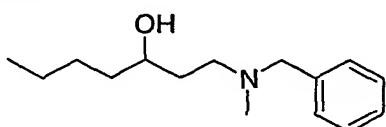
15   Add sodium borohydride (1.29 g, 34.1 mmol) to a stirred solution of 1-(benzyl-methyl-amino)-heptan-3-one (2.41 g, 10 mmol) and methanol (45ml) at 0 °C under N_2. Stir the reaction for 1 hr at 0 °C. Quench the reaction with water at 0 °C and concentrate under reduced pressure. Dissolve the residue in ethyl acetate, wash with brine and dry over sodium sulfate. Filter off and concentrate to give the crude compound. Purify the

20   compound by flash chromatography, eluting with 5% ethanol/0.5% ammonium hydroxide/chloroform to yield 2.22 g (91%) of 1-(benzyl-methyl-amino)-heptan-3-ol: mass spectrum (ion spray) m/z = 236 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.34-7.23 (m, 5H), 3.76-3.72 (m, 1H), 3.53 (dd, 2H), 2.80-2.73 (m, 1H), 2.59-2.53 (m, 1H), 2.21 (s, 3H), 1.68-1.62 (m, 1H), 1.57-1.29 (m, 7H), 0.91 (t, 3H).

25

1-(Benzyl-methyl-amino)-hexan-3-ol
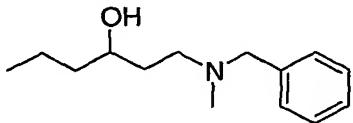
-71-

Using a method similar to that for 1-(Benzyl-methyl-amino)-heptan-3-ol, 1-(benzyl-methyl-amino)-hexan-3-one affords the title compound: mass spectrum (ion spray) m/z = 222 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.34-7.24 (m, 5H), 3.79-3.73 (m, 1H), 3.55 (dd, 2H), 2.81-2.74 (m, 1H), 2.59-2.55 (m, 1H), 2.22 (s, 3H), 1.72-1.62 (m, 1H), 1.56-1.31 (m, 5H), 0.93 (t, 3H).

(4-Cyclohexyl-3-hydroxy-butyl)-methyl-carbamic acid *tert*-butyl ester
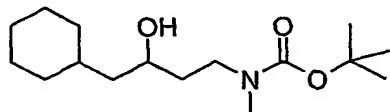
Using a method similar to that for 1-(Benzyl-methyl-amino)-heptan-3-ol, (4-cyclohexyl-3-oxo-butyl)-methyl-carbamic acid *tert*-butyl ester affords the title compound: ^{1}H NMR (CDCl$_3$) δ 3.90-3.80 (m, 1H), 3.62-3.52 (m, 1H), 2.88-2.96 (m, 1H), 2.83 (s, 3H), 1.80-1.60 (m, 6H), 1.56-1.32 (m, 3H), 1.47 (s, 9H), 1.31-1.08 (m, 4H), 0.98-0.77 (m,2H).


1-(Benzyl-methyl-amino)-5-methyl-hexan-3-ol
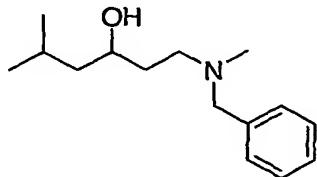
Add *N*-benzylmethylamine (1.50 mL, 11.6 mmol) to a stirred solution of 1,8-diazabicylco[5.4.0]undec-7-ene (0.0175 mL, 0.117 mmol) and anhydrous tetrahydrofuran (10 mL). Cool the solution to –15 °C under nitrogen. Add acrolein (0.78 mL, 11.7 mmol) slowly and stir for 30 minutes at –15 °C. Cool the reaction to –78 °C. Add isobutylmagnesium chloride (11.0 mL, 2.0 M solution in diethyl ether, 22.0 mmol) and stir for 1 hour at –78 °C. Quench the reaction with water at –78 °C and then pour the reaction into 100 mL of 1N sodium hydroxide/saturated sodium bicarbonate (1:1). Extract with ethyl acetate (3 X 100 mL), wash the ethyl acetate with brine (100 mL), and dry the ethyl acetate over sodium sulfate. Filter off the sodium sulfate and concentrate on a rotary evaporator to give the crude product. Purify the crude product by flash chromatography on silica gel eluting with 0.3% concentrated ammonium hydroxide/3% ethanol/chloroform to yield 1.0010 g (37%) of 1-(benzyl-methyl-amino)-5-methyl-hexan-3-ol: mass spectrum

(ion spray) m/z = 236.2(M+1); ^{1}H NMR (CDCl$_3$) δ 7.34-7.23 (m, 5H), 6.08 (br s, 1H), 3.86-3.80 (m, 1H), 3.62 (d, 1H), 3.43 (d, 1H), 2.79-2.73 (m, 1H), 2.58-2.53 (m, 1H), 2.21 (s, 3H), 1.83-1.40 (m, 4H), 1.16-1.09 (m, 1H), 0.91-0.89 (m, 6H).

5   **1-(Benzyl-methyl-amino)-4-methyl-pentan-3-ol**
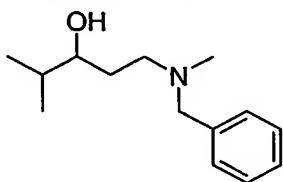
Using a method similar to that for 1-(Benzyl-methyl-amino)-5-methyl-hexan-3-ol, isopropylmagnesium chloride affords the title compound: mass spectrum (ion spray) m/z = 222.1(M+1); ^{1}H NMR (CDCl$_3$) δ 7.34-7.23 (m, 5H), 6.21 (br s, 1H), 3.63 (d, 1H),

10   3.51-3.47 (m, 1H), 3.42 (d, 1H), 2.81-2.74 (m, 1H), 2.59-2.54 (m, 1H), 2.19 (s, 3H), 1.72-1.47 (m, 3H), 0.95 (d, 3H), 0.89 (d, 3H).

[3-(Benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]-benzyl-methyl-amine
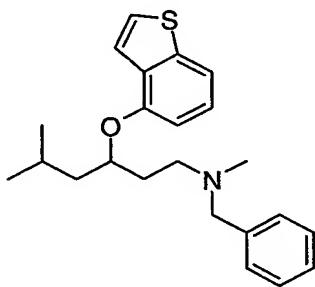
15   Add 1,1'-(azodicarbonyl)dipiperidine (1.6901 g, 6.698 mmol) to a stirred solution of 1-(benzyl-methyl-amino)-5-methyl-hexan-3-ol (1.0463 g, 4.445 mmol), 4-hydroxybenzo-thiophene (1.0027 g, 6.676 mmol), tributylphosphine (1.70 mL, 6.82 mmol), and anhydrous toluene (15 mL). Heat the reaction to 70 °C under nitrogen for 18 hours. Cool the reaction to room temperature and dilute with dichloromethane. Filter off the solid

20   material and concentrate the solvent off on a rotary evaporator to give the crude product. Purify the crude product by flash chromatography on silica gel eluting with 10% ethyl acetate /dichloromethane to yield 0.9674 g (59%) of [3-(benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]-benzyl-methyl-amine: mass spectrum (ion spray) m/z = 368.3(M+1); ^{1}H NMR (CDCl$_3$) δ 7.46-7.41 (m, 2H), 7.29-7.21 (m, 7H), 6.83 (d, 1H), 4.66-4.60 (m, 1H),

3.47 (s, 2H), 2.54-2.51 (m, 2H), 2.19 (s, 3H), 2.02-1.71 (m, 4H), 1.49-1.42 (m, 1H), 0.95 (d, 3H), 0.89 (d, 3H).

[3-(Benzo[*b*]thiophen-4-yloxy)-4-methyl-pentyl]-benzyl-methyl-amine
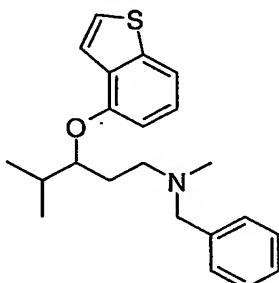
5

Using a method similar to that for [3-(Benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]-benzyl-methyl-amine, 1-(benzyl-methyl-amino)-4-methyl-pentan-3-ol affords the title compound: mass spectrum (ion spray) m/z = 354.3(M+1); ^{1}H NMR (CDCl$_3$) δ 7.49 (d, 1H), 7.43 (d, 1H), 7.31-7.19 (m, 7H), 6.85 (d, 1H), 4.48-4.44 (m, 1H), 3.51-3.43 (m, 2H),
10   2.61-2.48 (m, 2H), 2.19 (s, 3H), 2.11-1.85 (m, 3H), 1.03 (d, 3H), 0.99 (d, 3H).
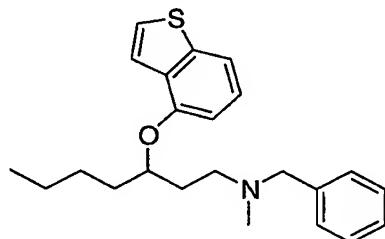
[3-(Benzo[*b*]thiophen-4-yloxy)-heptyl]-benzyl-methyl-amine

Using a method similar to that for [3-(Benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]-
15   benzyl-methyl-amine, 1-(benzyl-methyl-amino)-heptan-3-ol affords the title compound: mass spectrum (ion spray) m/z = 368 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.45-7.41 (m, 2H), 7.29-7.19 (m, 7H), 6.80 (d, 1H), 4.56-4.51 (m, 1H), 3.47 (s, 2H), 2.55 (t, 2H), 2.20 (s, 3H), 2.01-1.91 (m, 2H), 1.90-1.63 (m, 3H), 1.45-1.29 (m, 4H), 0.89 (t, 3H).

20   ## [3-(Benzo[*b*]thiophen-4-yloxy)-hexyl]-benzyl-methyl-amine

-74-
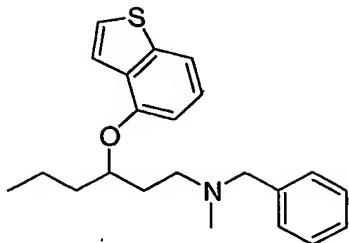
Using a method similar to that for [3-(Benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]-benzyl-methyl-amine, 1-(benzyl-methyl-amino)-hexan-3-ol affords the title compound: mass spectrum (ion spray) m/z = 354 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.45-7.41 (m, 2H),
7.29-7.20 (m, 7H), 6.81 (d, 1H), 4.58-4.53 (m, 1H), 3.47 (s, 2H), 2.54 (t, 2H), 2.20 (s, 3H), 2.03-1.90 (m, 2H), 1.88-1.60 (m, 2H), 1.53-1.38 (m, 2H), 0.93 (t, 3H).

[3-(Benzo[*b*]thiophen-4-yloxy)-4-cyclohexyl-butyl]-methyl-carbamic acid *tert*-butyl ester
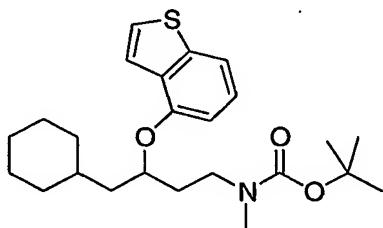
Using a method similar to that for [3-(Benzo[*b*]thiophen-4-yloxy)-5-methyl-hexyl]- ·
benzyl-methyl-amine, (4-cyclohexyl-3-hydroxy-butyl)-methyl-carbamic acid *tert*-butyl ester affords the title compound: mass spectrum (ion spray) m/z = 418 (M+1); ^{1}H NMR (CDCl$_3$) δ 7.49-7.47 (d, 1H), 7.43-7.41 (d, 1H), 7.31-7.30 (d, 1H), 7.24 (t, 1H), 6.72 (d, 1H), 4.59-4.52 (m, 1H), 3.34-3.27 (m, 2H), 2.80 (s, 3H), 1.98-0.92 (m, 24).

R,R)-(3-Propyl-oxiranyl)-methanol

Add trans-2-hexen-1-ol (12 mL, 102 mmol), (-)-diethyl tartrate (2.1 mL, 12.3 mmol), and Ti(O^{i}Pr)$_4$ (3.0 mL, 10.2 mmol) to a cooled (-20°C) solution of activated, dried, crushed 4Å molecular sieves (50 g) in dichloromethane (700 mL). After 30 minutes, add a dry [JACS, 1987, *109*, 5765] solution of *t*BuOOH in dichloromethane (~5M in dichloromethane, 57 mL, 285 mmol). Stir for 4 hours at –20°C and filter the solids. Add

700 mL of 15% L-tartartic acid to the filtrate and stir for 20 minutes. Separate the layers, extract the aqueous layer with dichloromethane, and concentrate the combined organic extracts *in vacuo*. Add 300 mL diethyl ether to residue and cool to 0°C. Add cool (0°C) 15% NaOH, stir for 15 minutes, separate, and extract aqueous layer with diethyl ether.

5   Wash the organic layer with aqueous saturated sodium chloride, dry over anhydrous MgSO$_4$, filter, and concentrate *in vacuo*. Purify on silica gel eluting with 0-50% EtOAc/hexanes to give (*R,R*)-(3-propyl-oxiranyl)-methanol (5.69 g, 48%). ^{1}H NMR (CDCl$_3$) δ 3.97-3.88 (m, 1H), 3.68-3.59 (m, 1H), 3.00-2.91 (m, 2H), 1.83-1.68 (m, 1H), 1.61-1.41 (m, 4H), 0.97 (t, 3H). A similarly carried out Sharpless asymmetric

10   epoxidation of trans-2-hexen-1-ol is described in *J. Org. Chem.* **1994**, *59*, 4461 and is reported to proceed in >95%ee by ^{1}H NMR analysis of the Mosher esters.

(2R,3S)-3-(3,5-Dichloro-phenoxy)-hexane-1,2-diol
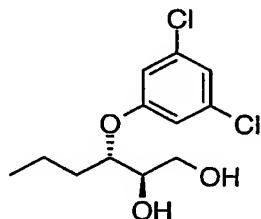
15

Add sodium hydroxide (1N, 9 mL) to a stirred solution of 3,5-dichlorophenol in THF (9 mL) followed by a solution of (*R,R*)-(3-propyl-oxiranyl)-methanol in THF (5 mL). Heat the reaction mixture at 75°C overnight, cool to room temperature, add water, brine and

20   dichloromethane, and separate the layers. The aqueous layer is extracted with dichloromethane, the combined organic extracts are washed successively with 1N sodium hydroxide and water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. Analysis of the crude reaction mixture by ^{1}H NMR reveals a ~2:1 mixture of the C-3:C-1 adducts. Purification of the crude residue on silica gel eluting

25   with 0-25 % EtOAc/hexanes gives the major title compound (332 mg, 26%). ^{1}H NMR (DMSO-d6): 0.86 (dd, 3H, J = 4, 4 Hz), 1.21-1.48 (m, 2H), 1.52-1.72 (m, 2H), 3.28-3.45 (m, 2H), 3.60-3.67 (m, 2H), 4.37-4.44 (m, 1H), 4.69 (dd, 1H, *J* = 6, 6 Hz), 4.88 (d, 1H, J = 5 Hz), 7.01-7.10 (m, 3H).

(2R,3S)-3-(2,4-Dichloro-phenoxy)-hexane-1,2-diol
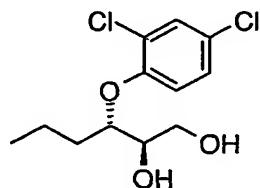
5   Using a method similar to that for described for the preparation of (2R,3S)-3-(3,5-
dichloro-phenoxy)-hexane-1,2-diol, the title compound is obtained as a powder (317 mg,
33%). ^{1}H NMR (DMSO-d6): 0.85 (dd, 3H, J = 7, 7 Hz), 1.23-1.50 (m, 2H), 1.56-1.77 (m,
2H), 3.35-3.50 (m, 2H), 3.63-3.70 (m, 2H), 4.38-4.44 (m, 1H), 4.65 (dd, 1H, J = 6, 6 Hz),
4.89 (d, 1H, J = 5 Hz), 7.20 (d, 1H, J = 9 Hz), 7.31 (dd, 1H, J = 9, 3 Hz), 7.51 (d, 1H, J =
10   3 Hz).

(2S, 3S)-Toluene-4-sulfonic acid 3-(3,5-dichloro-phenoxy)-2-hydroxy-hexyl ester
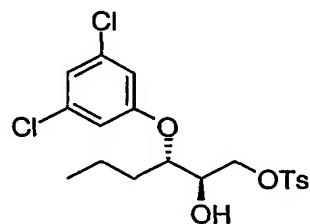
15

Add p-toluenesulfonyl chloride (339 mg, 1.78 mmol, 1.5 equiv.) to a cold (0°C) stirred
solution of (2R,3S)-3-(2,4-dichloro-phenoxy)-hexane-1,2-diol (332 mg, 1.19 mmol, 1
equiv.) and stir at 0°C for 6 hr before slowly warming to room temperature overnight.
Add dichloromethane and 1 N hydrochloric acid, separate the layers, extract the aqueous
20   layer with dichloromethane (three times), washed the combined organic extracts with 1N
hydrochloric acid, dry over anhydrous magnesium sulfate, filter, and concentrate under
reduced pressure. Purification of the crude residue on silica gel eluting with 0-15 %
EtOAc/hexanes gives the title compound as a colorless oil (306 mg, 59%). ^{1}H NMR
(CD$_3$OD): 0.86 (dd, 3H, J = 7, 7 Hz), 1.23-1.47 (m, 2H), 1.63-1.71 (m, 2H), 2.42 (s, 3H)
25   3.83-3.88 (m, 1H), 4.00 (dd, 1H, J = 10, 5 Hz), 4.15 (dd, 1H, J = 10, 4 Hz), 4.29-4.37 (m

-77-

1H), 6.79 (d, 1H, J = 2 Hz), 6.98 (dd, 1H, J = 2 Hz), 7.32 (d, 1H, J = 8 Hz), 7.67 (dd, 1H, J = 8 Hz).

(2S, 3S)-Toluene-4-sulfonic acid 3-(2,4-dichloro-phenoxy)-2-hydroxy-hexyl ester

5
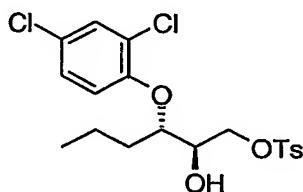
Using a method similar to that for described for the preparation of (2S, 3S)-toluene-4-sulfonic acid 3-(3,5-dichloro-phenoxy)-2-hydroxy-hexyl ester, the title compound is

10   obtained as a colorless viscous oil (266 mg, 55%). ^{1}H NMR (CDCl$_3$): 0.90 (dd, 3H, J = 7, 7 Hz), 1.30-1.52 (m, 2H), 1.57-1.80 (m, 2H), 2.44 (s, 3H) 3.99-4.06 (m, 1H), 4.17 (dd, 1H, J = 10, 6 Hz), 4.25-4.36 (m 2H), 6.87 (d, 1H, J = 9 Hz), 7.14 (d, 1H, J = 9 Hz), 7.27-7.39 (m, 2H), 7.73 (d, 1H, J = 8 Hz).

15   ## EXAMPLE 1

[3-(Benzo[b]thiophen-4-yloxy)-5-methyl-hexyl]-methyl-amine
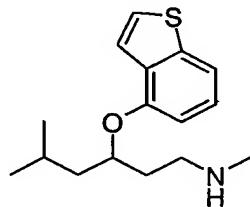
Add 1-chloroethylchloroformate (0.58 mL, 5.38 mmol) to a stirred solution of [3-
(benzo[b]thiophen-4-yloxy)-5-methyl-hexyl]-benzyl-methyl-amine (0.9619 g, 2.62 mmol)

20   and 1,2-dichloroethane (25 mL). Heat the reaction to reflux under nitrogen for 1.5 hours.
Add methanol (30 mL) and heat the reaction at reflux for 30 minutes. Cool the reaction to
room temperature and make it basic using 2M ammonia in methanol. Concentrate on a
rotary evaporator to give the crude product. Purify the crude product by flash
chromatography on silica gel eluting with 0.5% concentrated ammonium hydroxide/5%

25   ethanol/chloroform to yield 0.6303 g (87%) of [3-(benzo[b]thiophen-4-yloxy)-5-methyl-
hexyl]-methyl-amine: mass spectrum (ion spray) m/z = 278.2(M+1); HPLC (90/5/5 to

-78-

0/95/5 water/acetonitrile/5% formic acid in water) XTerra MS C_{18} 2.1mm X 50mm X 3.5micron: Retention time: 2.52 minutes, Purity: >99.9%.

EXAMPLE 2

5   [3-(Benzo[*b*]thiophen-4-yloxy)-4-methyl-pentyl]-methyl-amine
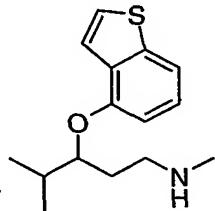
Using a method similar to example 1, [3-(benzo[*b*]-thiophen-4-yloxy)-4-methyl-pentyl]-benzyl-methyl-amine affords the title compound: mass spectrum (ion spray) m/z = 264.1(M+1); HPLC (90/5/5 to 0/95/5 water/acetonitrile/5% formic acid in water) XTerra

10   MS C_{18} 2.1mm X 50mm X 3.5micron: Retention time: 2.32 minutes, Purity: >99.9%.

EXAMPLE 3

[3-(Benzo[*b*]thiophen-4-yloxy)-heptyl]-methyl-amine
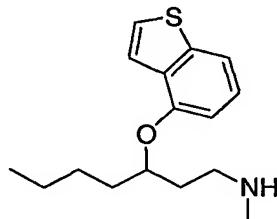
15   Using a method similar to example 1, [3-(benzo[*b*]thiophen-4-yloxy)-heptyl]-benzyl-methyl-amine affords the title compound: mass spectrum (ion spray): m/z = 278 (M+1); HPLC (90/5/5 to 0/95/5 water/acetonitrile/5% formic acid) Xterra MS C_{18} 2.1mm x 3.5mm: Retention time 2.60 minutes, Purity 100%.

20   **EXAMPLE 4**

[3-(Benzo[*b*]thiophen-4-yloxy)-hexyl]-methyl-amine

-79-
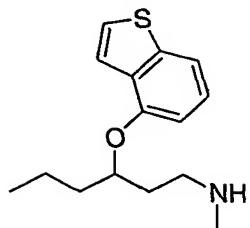
Using a method similar to example 1, [3-(benzo[b]thiophen-4-yloxy)-hexyl]-benzyl-methyl-amine affords the title compound: mass spectrum (ion spray) m/z = 264 (M+1); HPLC (90/5/5 to 0/95/5 water/acetonitrile/5% formic acid) Xterra MS C_{18} 2.1mm X

5   50mm X 3.5micron: Retention time 2.31 minutes, Purity 100%.

EXAMPLE 5

[3-(Benzo[b]thiophen-4-yloxy)-4-cyclohexyl-butyl]-methyl-amine
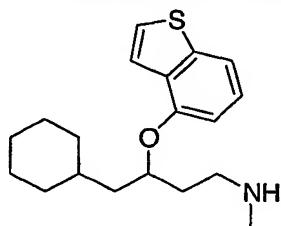
10   Add trifluoroacetic acid (5.5 ml, 71 mmol) to a stirred solution of [3-(benzo[b]thiophen-4-yloxy)-4-cyclohexyl-butyl]-methyl-carbamic acid tert-butyl ester (1.49 g, 3.6 mmol) and dichloromethane (80 ml) at 0 °C under N_2. Stir for 5 minutes at 0 °C and allow the reaction to reach room temperature. Stir for 90 minutes, add sodium bicarbonate. Partition between sodium bicarbonate and dichloromethane, wash the combined dichloromethane

15   layers with brine, dry over sodium sulfate. Filter and concentrate to give the crude compound. Purify the compound by flash chromatography, eluting with 3% ethanol/0.3% ammonium hydroxide/chloroform to yield 0.511 g (45%) of the title compound: mass spectrum (ion spray) m/z = 318 (M+1); HPLC (90/5/5 to 0/95/5 water/acetonitrile/5% formic acid) Xterra MS C_{18} 2.1mm X 50mm X 3.5micron: Retention time 3.08 minutes,

20   Purity 100%.

EXAMPLE 6

### 3-(Benzo[b]thiophen-4-yloxy)-4-cyclohexyl-N-methylbutan-1-amine hydrochloride

a)

-80-
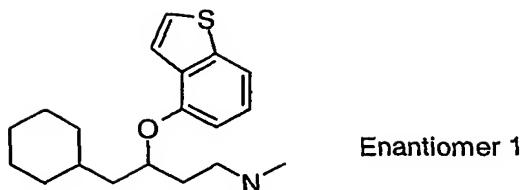
Enantiomer 1

Separate the racemic mixture of [3-(benzo[*b*]thiophen-4-yloxy)-4-cyclohexyl-butyl]-methyl-amine into the two enantiomers by chiral chromatography on a 15 cm x 4.6 mm i.d Chiralpak AD-H(3673) using 95% heptane/ 5% ethanol and 0.2% diethylamine as the mobile phase at a rate of 1.0 ml/min. Add dropwise with stirring 1M hydrochloric acid in diethyl ether (0.52 ml, 0.52 mmol, 1 eq) to a solution of the free base of the first eluting enantiomer (166 mg, 0.52 mmol, 1 eq) in diethyl ether (20 ml). Cool to 0 °C for several hours, filter the solid off and dry under vacuum to give the compound as a white solid (128 mg, 69%). ^{1}H NMR (300MHz, CD$_3$OD) δ 7.43-7.32 (3H, m, ArH), 7.18 (1H, t, J = 8.0 Hz, ArH), 6.81 (1H, d, J = 7.5 Hz, ArH), 4.76-4.61 (1H, m, 4-CH), 3.17-2.98 (2H, m, 2-CH$_2$), 2.58 (3H, s, NHCH_3), 2.22-1.89 (2H, m, 3-CH$_2$), 1.82-0.74 (13H, m, 6 x CH$_2$ and CH). LCMS m/z 318.1 [M+H].

b)
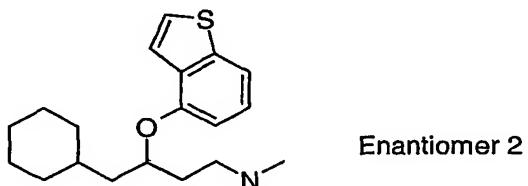
Enantiomer 2

Prepare the hydrochloride salt of the free base of the second eluting enantiomer (143 mg, 0.45 mmol) as described above to give the compound as a white solid (98 mg, 61%). ^{1}H NMR (300MHz, CD$_3$OD) δ 7.54-7.44 (3H, m, ArH), 7.30 (1H, t, J = 8.0 Hz, ArH), 6.93 (1H, d, J = 8.1 Hz, ArH), 5.00-4.73 (1H, m, 4-CH), 3.31-3.07 (2H, m, 2-CH$_2$), 2.69 (3H, s, NHCH_3), 2.31-1.99 (2H, m, 3-CH$_2$), 1.90-0.87 (13H, m, 6 x CH$_2$ and CH). LCMS m/z 318.1 [M+H].

EXAMPLE 7
3-(Benzo[*b*]thiophen-4-yloxy)-N,4-dimethylpentan-1-amine hydrochloride

a)

-81-
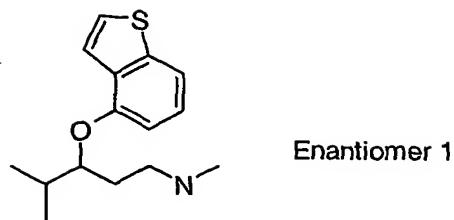
Enantiomer 1

Separate the racemic mixture of [3-(benzo[*b*]thiophen-4-yloxy)-4-methyl-pentyl]-methyl-amine into the two enantiomers by chiral chromatography on a Chiralcel-AD-H(15cm) using 95% ethanol/ 5% isopropanol and 0.2% diethylamine as the mobile phase at a rate of 0.5 ml/min. Using a method similar to example 6, prepare the hydrochloride salt of the free base of the first eluting enantiomer (176 mg, 0.63 mmol) to give a white solid (159 mg, 79%). ^{1}H NMR (300MHz, CD$_3$OD) δ 7.56-7.45 (3H, m, ArH), 7.29 (1H, t, J = 8.0 Hz, ArH), 6.93 (1H, d, J = 8.7 Hz, ArH), 4.59-4.49 (1H, m, 4-CH), 3.25-3.10 (2H, m, 2-CH$_2$), 2.69 (3H, s, NHCH$_3$), 2.24-2.06 (2H, m, 3-CH$_2$) and 1.12-0.98 (6H, m, 2 x CH$_3$). LCMS m/z 264.1 [M+H].

b)
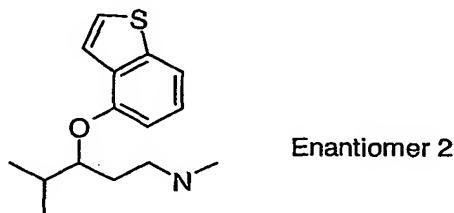
Enantiomer 2

Prepare the hydrochloride salt of the free base of the second eluting enantiomer (104 mg, 0.39 mmol) as described above to give the compound as a white solid (70 mg, 59%). ^{1}H NMR (300MHz, CD$_3$OD) δ 7.54-7.45 (3H, m, ArH), 7.29 (1H, t, J = 7.9 Hz, ArH), 6.93 (1H, d, J = 7.9 Hz, ArH), 4.64-4.46 (1H, m, 4-CH), 3.25-3.09 (2H, m, 2-CH$_2$), 2.69 (3H, s, NHCH$_3$), 2.26-2.04 (2H, m, 3-CH$_2$) and 1.12-0.96 (6H, m, 2 x CH$_3$). LCMS m/z 264.1 [M+H].

EXAMPLE 8

3-(Benzo[*b*]thiophen-4-yloxy)-N-methylhexan-1-amine hydrochloride

a)

-82-
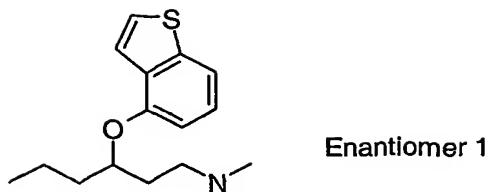
Enantiomer 1

Separate the racemic mixture of [3-(benzo[*b*]thiophen-4-yloxy)-hexyl]-methyl-amine into the two enantiomers by chiral chromatography on a 15 cm x 4.6 mm i.d Chiralpak AD-H(3687) using 95% Heptane/ 5% isopropanol and 0.2% diethylamine as the mobile phase at a rate of 0.5 ml/min. Prepare the hydrochloride salt of the free base as described in example 6 to give the compound as a white solid (52 mg, 53%). ^{1}H NMR (300MHz, CD$_3$OD) δ 7.43-7.33 (3H, m, ArH), 7.18 (1H, t, J = 8.3 Hz, ArH), 6.81 (1H, d, J = 7.5 Hz, ArH), 4.67-4.51 (1H, m, 4-CH), 3.17-2.91 (2H, m, 2-CH$_2$), 2.59 (3H, s, NHC$_3$), 2.17-1.95 (2H, m, 3-CH$_2$), 1.81-1.59 (2H, m, CH$_2$), 1.58-1.24 (2H, m, CH$_2$) and 0.93-0.75 (3H, m, CH$_3$). LCMS m/z 264.1 [M+H].

b)
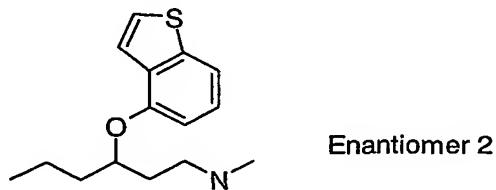
Enantiomer 2

Prepare the hydrochloride salt of the free base of the second eluting enantiomer (128 mg, 0.48 mmol) as described above to give a white solid (90 mg, 62%): ^{1}H NMR (300MHz, CD$_3$OD) δ 7.41-7.31 (3H, m, ArH), 7.18 (1H, t, J = 8.2 Hz, ArH), 6.81 (1H, d, J = 7.9 Hz, ArH), 4.65-4.49 (1H, m, 4-CH), 3.20-2.96 (2H, m, 2-CH$_2$), 2.59 (3H, s, NHC$_3$), 2.15-1.97 (2H, m, 3-CH$_2$), 1.79-1.51 (2H, m, CH$_2$), 1.49-1.27 (2H, m, CH$_2$) and 0.91-0.77 (3H, m, CH$_3$). LCMS m/z 264.1 [M+H].

EXAMPLE 9

**3-(Benzo[*b*]thiophen-4-yloxy)-N-methylheptan-1-amine hydrochloride**

a)

-83-
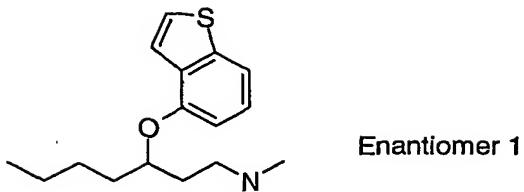
Enantiomer 1

Separate the racemic mixture [3-(benzo[*b*]thiophen-4-yloxy)-heptyl]-methyl-amine into their two enantiomers by chiral chromatography on a Chiralcel OD(3641) using 95% Heptane/ 5% ethanol and 0.2% diethylamine as the mobile phase at a rate of 0.5 ml/min.

5   Add dropwise a solution of hydrochloric acid in diethyl ether (2M) to a solution of the free base of the first eluting enantiomer (100 mg, 0.36 mmol) in EtOH (5 mL). Stir the mixture at room temperature for 10 minutes and evaporate all volatiles *in vacuo*. Shake the resulting amorphous solid in diethyl ether until a crystalline solid is formed. Filter off the solid and dry under vacuum to give the compound as a brown solid (90 mg, 77 %). ^{1}H

10   NMR (300MHz, DMSO-d_6) δ 8.85 (2H, bs), 7.66-7.64 (1H, m), 7.56-7.53 (1H, m), 7.46-7.44 (1H, m), 7.32-7.27 (1H, m), 6.97-6.94 (1H, m), 4.67-4.64 (1H, m), 3.37 (3H, s), 3.03-3.00 (2H, m), 2.10-2.03 (2H, m), 1.68-1.59(2H, m), 1.39-1.31 (4H, m), 0.87-0.82 (3H, m). LCMS m/z 278 [M+H].

15   b)
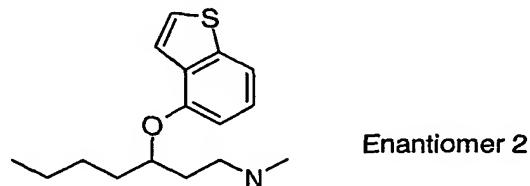
Enantiomer 2

Prepare the hydrochloride salt of the free base of the second eluting enantiomer (118 mg, 0.42 mmol) as described above to give a brown solid (40 mg, 34 %). ^{1}H NMR (300MHz, DMSO-d_6) δ 8.79 (2H, bs), 7.66-7.64 (1H, m), 7.56-7.53 (1H, m), 7.46-7.44b (1H, m),

20   7.32-7.27 (1H, m), 6.97-6.94 (1H, m), 4.67-4.63 (1H, m), 3.32 (3H, s), 3.03-3.01 (2H, m), 2.09-2.02 (2H, m), 1.68-1.59 (2H, m), 1.39-1.31 (4H, m) and 0.87-0.82 (3H, m). LCMS m/z 278 [M+H].

EXAMPLE 10

25   **(2S,3S)-3-(3,5-Dichloro-phenoxy)-1-methylamino-hexan-2-ol hydrochloride**

-84-
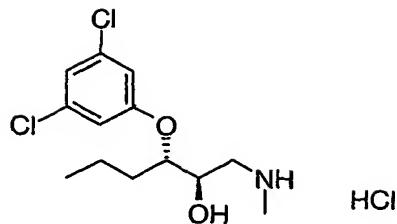
Add methyl amine (5 mL, 40%wt in water) to a solution of (2S, 3S)-toluene-4-sulfonic acid 3-(3,5-dichloro-phenoxy)-2-hydroxy-hexyl ester (306 mg, 0.706 mmol) in 1,4-dioxane (10 mL) in a heavy walled screw top sealed tube, seal the tube, and heat at 50°C overnight. The mixture is cooled and concentrated under reduced pressure. Purification by medium pressure liquid chromatography eluting with 0-10% of 2N NH₃/MeOH in dichloromethane is followed by HCl salt formation by dissolving in methanol (10 mL), adding solid ammonium chloride (32 mg, 0.598 mmol) and sonicating for 15 minutes. The mixture is concentrated under reduced pressure and the residue is dissolved in water, frozen at –78°C, and freeze dried to afford the title compound as a colorless solid (170 mg, 73%): δ_H (CD₃OD, 400 MHz): 0.94 (dd, 3H, J = 7, 7 Hz), 1.31-1.55 (m, 2H), 1.60-1.79 (m, 2H), 2.72 (s, 3H), 3.11 (dd, 1H, J = 13, 10 Hz), 3.22 (dd, 1H, J = 13, 3 Hz), 3.97-4.05 (m 1H), 4.37-4.45 (m, 1H), 7.00-7.04 (m, 3H). Exact Mass cacld. for $C_{13}H_{19}O_2NCl_2$ (M+ free base): 292.0871; found: 292.0879.

EXAMPLE 11

(2S,3S)- 3-(2,4-Dichloro-phenoxy)-1-methylamino-hexan-2-ol tartrate
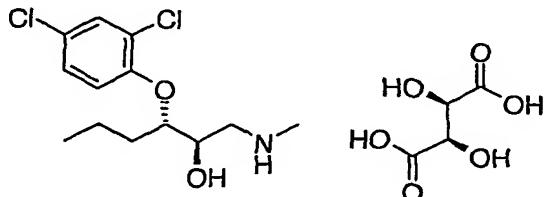
Add methyl amine (3 mL, 40%wt in water) to a solution of (2S, 3S)-toluene-4-sulfonic acid 3-(2,4-dichloro-phenoxy)-2-hydroxy-hexyl ester (266 mg, 0.614 mmol) in 1,4-dioxane (6 mL) in a heavy walled screw top sealed tube, seal the tube, and heat at 50°C overnight. The mixture is cooled and concentrated under reduced pressure. Purification

-85-

by medium pressure liquid chromatography eluting with 0-10% of 2N NH$_3$/MeOH in dichloromethane is followed by L-tartrate salt formation by dissolving in methanol, adding L-tartrate (65.6 mg, 0.437 mmol) and sonicating. The mixture is concentrated under reduced pressure and the residue is dissolved in water, frozen at –78°C, and freeze dried to afford the title compound as a colorless solid (172.8 mg, 64%): δ$_H$ (CD$_3$OD, 400 MHz): 0.94 (dd, 3H, J = 8, 8 Hz), 1.34-1.55 (m, 2H), 1.64-1.82 (m, 2H), 2.73 (s, 3H), 3.17 (dd, 1H, J = 12, 10 Hz), 3.33 (dd, 1H, J = 12, 3 Hz), 4.06-4.13 (m 1H), 4.40-4.48 (m, 3H), 7.17 17 (d, 1H, J = 9 Hz), 7.25 17 (dd, 1H, J = 9, 3 Hz), 7.40 17 (d, 1H, J = 3 Hz). Mass spectrum (ion spray): m/z = 292 (M+).

The compounds of the present invention may be used as medicaments in human or veterinary medicine. The compounds may be administered by various routes, for example, by oral or rectal routes, topically or parenterally, for example by injection, and are usually employed in the form of a pharmaceutical composition.

Such compositions may be prepared by methods well known in the pharmaceutical art and normally comprise at least one active compound in association with a pharmaceutically acceptable diluent or carrier. In making the compositions of the present invention, the active ingredient will usually be mixed with a carrier or diluted by a carrier, and/or enclosed within a carrier which may, for example, be in the form of a capsule, sachet, paper or other container. Where the carrier serves as a diluent, it may be solid, semi-solid, or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the composition may be in the form of tablets, lozenges, sachets, cachets, elixirs, suspensions, solutions, syrups, aerosol (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, injection solutions and suspensions and sterile packaged powders.

Some examples of suitable carriers are lactose, dextrose, vegetable oils, benzyl alcohols, alkylene glycols, polyethylene glycols, glycerol triacetate, gelatin, carbohydrates such as starch and petroleum jelly, sucrose sorbitol, mannitol, starches, gum acacia,calcium phosphate, alginates, tragacanth, gelatin, syrup, methyl cellulose, methyl- and propyl-

-86-

hydrobenzoate, talc, magnesium stearate and mineral oil. The compounds of formula (I)
can also be lyophilized and the lyophilizates obtained used, for example, for the
production of injection preparations. The preparations indicated can be sterilized and/or
can contain auxiliaries such as lubricants, preservatives, stabilizers and/or wetting agents,

5   emulsifiers, salts for affecting the osmotic pressure, buffer substances, colourants,
flavourings and/or one or more further active compounds, e.g. one or more vitamins.
Compositions of the invention may be formulated so as to provide, quick, sustained or
delayed release of the active ingredient after administration to the patient by employing
procedures well known in the art.

10

The compositions are preferably formulated in a unit dosage form, each dosage containing
from about 5 to about 500 mg, more usually about 25 to about 300 mg, of the active
ingredient. The term "unit dosage form" refers to physically discrete units suitable as
unitary doses for human subjects and other mammals, each unit containing a

15   predetermined quantity of active material calculated to produce the desired therapeutic
effect, in association with a suitable pharmaceutical carrier.

The pharmacological profile of the present compounds may be demonstrated as follows.
All of the exemplified compounds above have been found to exhibit K_i values less than

20     $1\mu M$ at the serotonin and norepinephrine transporters as determined using the scintillation
proximity assays described below. Furthermore, all of the exemplified compounds above
have been found to exhibit a K_i value less than 100nM at the serotonin transporter and/or
a K_i value less than 100nM at the norepinephrine transporter as determined using the
scintillation proximity assays described below. Preferred compounds of the present

25   invention are those which exhibit a K_i value less than 100nM (preferably less than 50nM)
at the serotonin transporter and a K_i value less than 100nM (preferably less than 50nM) at
the norepinephrine transporter as determined using the scintillation proximity assays
described below. Furthermore, all of the exemplified compounds above have been found
to selectively inhibit the serotonin and norepinephrine transporters relative to the

30   dopamine transporter. Preferred compounds of the present invention selectively inhibit the
serotonin and norepinephrine transporters relative to the dopamine transporter by a factor
of at least five using the scintillation proximity assays as described below.

-87-

Generation of stable cell-lines expressing the human dopamine, norepinephrine and serotonin transporters

Standard molecular cloning techniques were used to generate stable cell-lines expressing the human dopamine, norepinephrine and serotonin transporters. The polymerase chain reaction (PCR) was used in order to isolate and amplify each of the three full-length cDNAs from an appropriate cDNA library. Primers for PCR were designed using the following published sequence data:

Human dopamine transporter: GenBank M95167. Reference: Vandenbergh DJ, Persico AM and Uhl GR. *A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic TaqI RFLPs.* Molecular Brain Research (1992) volume 15, pages 161-166.

Human norepinephrine transporter: GenBank M65105. Reference: Pacholczyk T, Blakely, RD and Amara SG. *Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter.* Nature (1991) volume 350, pages 350-354.

Human serotonin transporter: GenBank L05568. Reference: Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V and Blakely RD. *Antidepressant- and cocaine-sensitive human serotonin transporter: Molecular cloning, expression, and chromosomal localization.* Proceedings of the National Academy of Sciences of the USA (1993) volume 90, pages 2542-2546.

The PCR products were cloned into a mammalian expression vector (eg pcDNA3.1 (Invitrogen)) using standard ligation techniques. The constructs were then used to stably transfect HEK293 cells using a commercially available lipofection reagent (Lipofectamine™ – Invitrogen) following the manufacture's protocol.

Scintillation proximity assays for determining the affinity of test ligands at the norepinephrine and serotonin transporters.

The compounds of the present invention are norepinephrine and serotonin reuptake inhibitors, and possess excellent activity in, for example, a scintillation proximity assay

-88-

(e.g. J. Gobel, D.L. Saussy and A. Goetz, J. Pharmacol. Toxicolo. (1999), 42, 237-244). Thus ^{3}H-nisoxetine binding to norepinephrine re-uptake sites in a cell line transfected with DNA encoding human norepinephrine transporter binding protein and similarly ^{3}H-citalopram binding to serotonin re-uptake sites in a cell line transfected with DNA

5   encoding human serotonin transporter binding protein have been used to determine the affinity of ligands at the norepinephrine and serotonin transporters respectively.

Norepinephrine Binding Assay

Membrane Preparation:

10   Cell pastes from large scale production of HEK-293 cells expressing cloned human norepinephrine transporters were homogenized in 4 volumes 50mM Tris-HCl containing 300mM NaCl and 5mM KCl, pH 7.4. The homogenate was centrifuged twice (40,000g, 10min, 4°C) with pellet re-suspension in 4 volumes of Tris-HCl buffer containing the above reagents after the first spin and 8 volumes after the second spin. The suspended

15   homogenate was centrifuged (100g, 10min, 4°C) and the supernatant kept and re-centrifuged (40,000g, 20min, 4°C). The pellet was resuspended in Tris-HCl buffer containing the above reagents along with 10%w/v sucrose and 0.1mM phenylmethylsulfonyl fluoride (PMSF). The membrane preparation was stored in aliquots (1ml) at −80°C until required. The protein concentration of the membrane preparation

20   was determined using a bicinchoninic acid (BCA) protein assay reagent kit (available from Pierce).

[^{3}H]-Nisoxetine Binding Assay:

Each well of a 96 well microtitre plate was set up to contain the following:

25   50μl   2nM [N-methyl-^{3}H]-Nisoxetine hydrochloride (70-87Ci/mmol, from NEN Life Science Products)

       75μl   Assay buffer (50mM Tris-HCl pH 7.4 containing 300mM NaCl and 5mM KCl)

       25μl   Test compound, assay buffer (total binding) or 10μM Desipramine HCl (non-specific binding)

30   50μl   Wheatgerm agglutinin coated poly (vinyltoluene) (WGA PVT) SPA Beads (Amersham Biosciences RPNQ0001) (10mg/ml)

       50μl   Membrane (0.2mg protein per ml)

−89−

The microtitre plates were incubated at room temperature for 10 hours prior to reading in a Trilux scintillation counter. The results were analysed using an automatic spline fitting programme (Multicalc, Packard, Milton Keynes, UK) to provide Ki values for each of the test compounds.

5

Serotonin Binding Assay

The ability of a test compound to compete with [^{3}H]-citalopram for its binding sites on cloned human serotonin transporter containing membranes has been used as a measure of test compound ability to block serotonin uptake via its specific transporter

10 (Ramamoorthy, S., Giovanetti, E., Qian, Y., Blakely, R., (1998) J. Biol. Chem. 273, 2458).

Membrane Preparation:

Membrane preparation is essentially similar to that for the norepinephrine transporter

15 containing membranes as described above. The membrane preparation was stored in aliquots (1ml) at −70°C until required. The protein concentration of the membrane preparation was determined using a BCA protein assay reagent kit.

[^{3}H]-Citalopram Binding Assay:

20 Each well of a 96 well microtitre plate was set up to contain the following:

50µl   2nM [^{3}H]-Citalopram (60-86Ci/mmol, Amersham Biosciences)

75µl   Assay buffer (50mM Tris-HCl pH 7.4 containing 150mM NaCl and 5mM KCl)

25µl   Diluted compound, assay buffer (total binding) or 100µM Fluoxetine (non-specific binding)

25 50µl   WGA PVT SPA Beads (40mg/ml)

50µl   Membrane preparation (0.4mg protein per ml)

The microtitre plates were incubated at room temperature for 10 hours prior to reading in a Trilux scintillation counter. The results were analysed using an automatic spline fitting programme (Multicalc, Packard, Milton Keynes, UK) to provide Ki (nM) values for each

30 of the test compounds.

Dopamine Binding Assay

-90-

The ability of a test compound to compete with [^{3}H]-WIN35,428 for its binding sites on human cell membranes containing cloned human dopamine transporter has been used as a measure of the ability of such test compounds to block dopamine uptake via its specific transporter (Ramamoorthy et al 1998 *supra*).

Membrane Preparation:

Is essentially the same as for membranes containing cloned human serotonin transporter as described above.

[^{3}H]-WIN35,428 Binding Assay:

Each well of a 96well microtitre plate was set up to contain the following:

50µl   4nM [^{3}H]-WIN35,428 (84-87Ci/mmol, from NEN Life Science Products)

75µl   Assay buffer (50mM Tris-HCl pH 7.4 containing 150mM NaCl and 5mM KCl)

25µl   Diluted compound, assay buffer (total binding) or 100µM Nomifensine (non-specific binding)

50µl   WGA PVT SPA Beads (10mg/ml)

50µl   Membrane preparation (0.2mg protein per ml.)

The microtitre plates were incubated at room temperature for 120 minutes prior to reading in a Trilux scintillation counter. The results were analysed using an automatic spline fitting programme (Multicalc, Packard, Milton Keynes, UK) to provide Ki values for each of the test compounds.

Formalin Paw Assay

The analgesic effect of compounds of the invention for the treatment of persistent nociceptive pain was demonstrated using the well-known "formalin test." The formalin test is a model of persistent nociceptive activation induced by tissue injury which can lead to central sensitization. (Shibata, M., Ohkubo, T., Takahashi, H., and Inoki, R., "Modified formalin test: Characteristic biphasic pain response," *Pain* (1989) 38: 347-352; and Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., and Hole, K., "The formalin test: an evaluation of the method," *Pain* (1992) 51:5-17.) The effect of compounds of the invention on formalin-induced paw-licking behavior in the rat was investigated as an

-91-

index of persistent nociceptive activation. In this test, the injection of formalin under the skin on the dorsal lateral surface of the hind paw of rats causes an immediate and intense increase in the spontaneous activity of C fiber afferents. This activation evokes a distinctly quantifiable behavior indicative of pain, such as licking of the injected paw.

5   The behavioral response to formalin is biphasic, with an early phase that is short lived, followed by an extended tonic response or late phase of persistent nociceptive activation. Mechanisms causing the late phase response, such as central sensitization of pain transmitting neurons, are currently believed to contribute to various types of persistent pains.

10

Male Sprague-Dawley rats (200-250g; Charles River, Portage, MI) were maintained at constant temperature and light (12h light/12h dark) for 4-7 days prior to the studies. Animals had free access to food and water at all times prior to the day of the experiment.

15   Scoring in the formalin test was performed according to Coderre *et al.*, 1993b and Abbott *et al.*, 1995. (Coderre T.J., Fundytus M.E., McKenna J.E., Dalal S. and Melzack R. "The formalin test: a validation of the weighted-scores method of the behavioral pain rating," *Pain*(1993b) **54**: 43-50; and Abbott F.V., Franklin K.B.J. and Westbrook R.F. "The formalin test: scoring properties of the first and second phases of the pain response in

20   rats," *Pain* (1995) **60**: 91-102.) The sum of time spent licking in seconds from time 0 to 5 minutes was considered the early phase while the late phase was taken as the sum of seconds spent licking from 15 to 40 minutes.

Data are presented as means with standard errors of means ($\pm$ SEM). Data were evaluated

25   by one-way analysis of variance (ANOVA) and the appropriate contrasts analyzed by Tukey's test and Dunnett "t' test for two-sided comparisons.

The preferred compounds of the present invention show good stability to the action of the CYP 2D6 enzyme. This is advantageous because it is likely to lead to improved

30   metabolic stability of the compounds.

-92-

Stability to the CYP 2D6 enzyme may be determined according to the assay described below:

In Vitro Determination of the Interaction of compounds with CYP2D6 in Human Hepatic Microsomes

Cytochrome P450 2D6 (CYP2D6) is a mammalian enzyme which is commonly associated with the metabolism of around 30% of pharmaceutical compounds. Moreover, this enzyme shows a genetic polymorphism with as a consequence a presence in the population of poor and normal metabolizers. A low involvement of CYP2D6 in the metabolism of compounds (i.e. the compound being a poor substrate of CYP2D6) is desirable in order to reduce any variability from subject to subject in the pharmacokinetics of the compound. Also, compounds with a low inhihibitor potential for CYP2D6 are desirable in order to avoid drug-drug interactions with co-administered drugs that are substrates of CYP2D6. Compounds may be tested both as substrates and as inhibitors of this enzyme by means of the following assays.

CYP2D6 substrate assay
Principle:

This assay determines the extent of the CYP2D6 enzyme involvement in the total oxidative metabolism of a compound in microsomes. Preferred compounds of the present invention exhibit less than 75% total metabolism via the CYP2D6 pathway.

For this in vitro assay, the extent of oxidative metabolism in human liver microsomes (HLM) is determined after a 30 minute incubation in the absence and presence of Quinidine, a specific chemical inhibitor of CYP2D6. The difference in the extent of metabolism in absence and presence of the inhibitor indicates the involvement of CYP2D6 in the metabolism of the compound.

Materials and Methods:

Human liver microsomes (mixture of 20 different donors, mixed gender) were acquired from Human Biologics (Scottsdale, AZ, USA). Quinidine and β–NADPH (β–Nicotinamide Adenine Dinucleotide Phosphate, reduced form, tetrasodium salt) were purchased from Sigma (St Louis, MO, USA). All the other reagents and solvents were of

analytical grade. A stock solution of the new chemical entity (NCE) was prepared in a mixture of Acetonitrile/Water to reach a final concentration of acetonitrile in the incubation below 0.5%.

5   The microsomal incubation mixture (total volume 0.1 mL) contained the NCE (4 μM), β-NADPH (1 mM), microsomal proteins (0.5 mg/mL), and Quinidine (0 or 2 μM) in 100 mM sodium phosphate buffer pH 7.4. The mixture was incubated for 30 minutes at 37 °C in a shaking waterbath. The reaction was terminated by the addition of acetonitrile (75 μL). The samples were vortexed and the denaturated proteins were removed by centrifugation. The amount of NCE in the supernatant was analyzed by liquid

10   chromatography /mass spectrometry (LC/MS) after addition of an internal standard. A sample was also taken at the start of the incubation (t=0), and analysed similarly.

Analysis of the NCE was performed by liquid chromatography /mass spectrometry. Ten μL of diluted samples (20 fold dilution in the mobile phase) were injected onto a Spherisorb CN Column, 5 μM and 2.1 mm x 100 mm (Waters corp.

15   Milford, MA, USA). The mobile phase consisting of a mixture of Solvent A/Solvent B, 30/70 (v/v) was pumped (Alliance 2795, Waters corp. Milford, MA, USA) through the column at a flow rate of 0.2 ml/minute. Solvent A and Solvent B were a mixture of ammonium formate 5.10^{-3} M pH 4.5/ methanol in the proportions 95/5 (v/v) and 10/90 (v/v), for solvent A and solvent B, respectively. The NCE and the internal standard were

20   quantified by monitoring their molecular ion using a mass spectrometer ZMD or ZQ (Waters-Micromass corp, Machester, UK) operated in a positive electrospray ionisation.

The extent of CYP2D6 involvement (% of CYP2D6 involvement) was calculated comparing the extent of metabolism in absence and in presence of quinidine in the incubation.

25   The extent of metabolism without inhibitor (%) was calculated as follows:

$$\frac{(\text{NCE response in samples without inhibitor})\text{time}0 - (\text{NCE response in samples without inhibitor})\text{time}30}{(\text{NCE response in samples without inhibitor})\text{time}0} \times 100$$

The extent of metabolism with inhibitor (%) was calculated as follows:

$$\frac{(\text{NCE response in samples without inhibitor})\text{time}0 - (\text{NCE response in samples with inhibitor})\text{time}30}{(\text{NCE response in samples without inhibitor})\text{time}0} \times 100$$

where the NCE response is the area of the NCE divided by the area of the internal

30   standard in the LC/MS analysis chromatogram, time0 and time30 correspond to the 0 and 30 minutes incubation time.

The % of CYP2D6 involvement was calculated as follows:

$$\frac{(\% \text{ extent of metabolism without inhibitor}) - (\% \text{ extent of metabolism with inhibitor})}{\% \text{ extent of metabolism without inhibitor}} \times 100$$

CYP2D6 inhibitor assay

Principle:

The CYP2D6 inhibitor assay evaluates the potential for a compound to inhibit CYP2D6. This is performed by the measurement of the inhibition of the bufuralol 1'-hydroxylase activity by the compound compared to a control. The 1'-hydroxylation of bufuralol is a metabolic reaction specific to CYP2D6. Preferred compounds of the present invention exhibit an IC_{50} higher than 6 μM for CYP2D6 activity, the IC_{50} being the concentration of the compound that gives 50 % of inhibition of the CYP2D6 activity.

Material and methods:

Human liver microsomes (mixture of 20 different donors, mixed gender) were acquired from Human Biologics (Scottsdale, AZ). β–NADPH was purchased from Sigma (St Louis, MO). Bufuralol was purchased from Ultrafine (Manchester, UK). All the other reagents and solvents were of analytical grade.

Microsomal incubation mixture (total volume 0.1 mL) contained bufuralol 10 μM, β–NADPH (2 mM), microsomal proteins (0.5 mg/mL), and the new chemical entity (NCE) (0, 5, and 25 μM) in 100 mM sodium phosphate buffer pH 7.4. The mixture was incubated in a shaking waterbath at 37 °C for 5 minutes. The reaction was terminated by the addition of methanol (75 μL). The samples were vortexed and the denatured proteins were removed by centrifugation. The supernatant was analyzed by liquid chromatography connected to a fluorescence detector. The formation of the 1'-hydroxybufuralol was monitored in control samples (0 μM NCE) and in the samples incubated in presence of the NCE. The stock solution of NCE was prepared in a mixture of Acetonitrile/Water to reach a final concentration of acetonitrile in the incubation below 1.0%.

The determination of 1'hydroxybufuralol in the samples was performed by liquid chromatograhy with fluorimetric detection as described below. Twenty five μL samples were injected onto a Chromolith Performance RP-18e column (100 mm x 4.6 mm) (Merck KGAa, Darmstadt, Germany). The mobile phase, consisting of a mixture of

solvent A and solvent B whose the proportions changed according the following linear

gradient, was pumped through the column at a flow rate of 1 ml/min:

Time (minutes)	Solvent A (%)	Solvent B (%)
0	65	35
2.0	65	35
2.5	0	100
5.5	0	100
6.0	65	35

5   Solvent A and Solvent B consisted of a mixture of 0.02 M potassium

dihydrogenophosphate buffer pH3/ methanol in the proportion 90/10 (v/v) for solvent A

and 10/90 (v/v) for solvent B. The run time was 7.5 minutes. Formation of 1'-

hydroxybufuralol was monitored by fluorimetric detection with extinction at λ 252 nm

and emission at λ 302 nm.

10   The IC_{50} of the NCE for CYP2D6 was calculated by the measurement of the

percent of inhibition of the formation of the 1'-hydroxybufuralol in presence of the NCE

compared to control samples (no NCE) at a known concentration of the NCE.

The percent of inhibition of the formation of the 1'-hydroxybufuralol is calculated

as follows:

15      $$\frac{(1\text{'-hydroxybufuralol formed without inhibitor}) - (1\text{'-hydroxybufuralol formed with inhibitor})}{(1\text{'-hydroxybufuralol area formed without inhibitor})} \times 100$$

The IC_{50} is calculated from the percent inhibition of the formation of the 1'-

hydroxybufuralol as follows (assuming competitive inhibition):

$$\frac{NCE \text{ Concentration} \times (100 - \text{Percent of inhibition})}{\text{Percent of inhibition}}$$

The IC_{50} estimation is assumed valid if inhibition is between 20% and 80%

20   (Moody GC, Griffin SJ, Mather AN, McGinnity DF, Riley RJ. 1999. Fully automated

analysis of activities catalyzed by the major human liver cytochrome P450 (CYP)

enzymes: assessment of human CYP inhibition potential. Xenobiotica, 29(1): 53-75).